UPTAKE AND REGENERATION OF DISSOLVED ORGANIC NITROGEN IN SOUTHEASTERN ALASKAN MARINE WATERS

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By

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UPTAKE AND REGENERATION OF DISSOLVED

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ALASKAN MARINE WATERS

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ABSTRACT

UPTAKE AND REGENERATION OF DISSOLVED ORGANIC NITROGEN IN SOUTHEASTERN ALASKAN MARINE WATERS

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The nutritive importance of dissolved organic nitrogen to phytoplankton populations was assessed in Auke Bay, Alaska (58°10'N, 134°40'W) during the period March 23 - June 6, 1968. Nitrate, ammonia, total dissolved organic nitrogen and dissolved free amino acids were monitored and related to phytoplankton growth. Total dissolved organic nitrogen ranged from <1 to >8 µg-atoms N/liter. Glycine and glutamic acid, the most abundant of the free amino acids, ranged from <0.02 µmoles/liter to 1.0 µmole/liter. Serine, aspartic acid, and valine were also common but usually at levels less than 0.02 µmoles/liter. Uptake rates for ^{15}N-labeled glycine and glutamic acid ranged from 0.001 to 0.002 µg-atom N/liter-hour-µg-atom particulate N, compared to 0.03 to 0.05 µg-atom N/liter-hour-µg-atom particulate N for nitrate and ammonia. Uptake experiments employing both ^{15}N- and ^{14}C- labeled glycine and glutamic acid showed that the nitrogen of glycine is preferentially incorporated, the carbon being respired, and the reverse is true for glutamic acid, the nitrogen presumably being released as ammonia. Phytoplankton supplied with 14 µg-atom NO_3-^{15}N/liter released approximately 10 percent of the ^{15}N back into the seawater as dissolved organic nitrogen within 48 hours. This value is probably conservative by virtue of the experimental procedure.

In general, dissolved organic nitrogen levels reflect phytoplankton growth in the euphotic zone. Below the euphotic zone, dissolved organic nitrogen is respired and ammonia accumulates. From the evidence obtained it was uncertain as to whether phytoplankton or bacteria were utilizing the organic carbon present below the euphotic zone.

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My sincere appreciation also goes to Mr. Peter Neas, skipper of the MAYBESO and voluntary scientific technician during our stations in Auke Bay and to Mrs. Dorothy Underwood who typed the original draft and offered numerous constructive criticisms.

Finally recognition goes to Dr. Donald W. Hood, who above all others, supplied the incentive to compile and complete this thesis.

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Chapter I

INTRODUCTION

a. Study Objectives and Limitations

Nitrogen, as one of the primary elements comprising all living organisms, often determines the productivity of biological communities through its availability to the primary producers therein. The nitrogen cycle within the sea has received extensive study and many of the complexities of the biological interactions with the inorganic fixed nitrogen have been described. The least understood and perhaps most intricate of the nitrogen pathways involve those concerned with the dissolved organic nitrogen fraction, and the purpose of this study may be broadly defined as an investigation into some aspects of dissolved organic nitrogen utilization by primary producers in the marine environment. In a specific sense, the following questions were used as guides in conducting the research program in the coastal waters of Southeastern Alaska.

1) What are the naturally occurring concentrations of dissolved organic nitrogen in seawater? How do these concentrations vary from nonproductive winter conditions to highly productive spring and summer conditions.

2) What are the ambient concentrations of such organic nitrogen metabolites as dissolved free amino acids? How do they vary in productive and nonproductive waters?

3) What are the relationships between dissolved organic nitrogen concentrations in seawater and the densities of phytoplankton populations? Is there evidence of pronounced excretion during rapid growth or senescence?

4) What are the uptake rates of specific organic metabolites at ambient levels? How do these uptake rates compare with those for nitrate and ammonia?

5) How long does it take for assimilated inorganic nitrogen to appear as dissolved organic nitrogen?

6) What is the fate of excreted organic nitrogen? How fast is it re-absorbed into the phytoplankton cells? How fast is it converted to inorganic forms of fixed nitrogen?

7) What are the overall ecological implications associated with dissolved organic nitrogen and the phytoplankton community?

To undertake a comprehensive and detailed analysis of what encompasses nearly the entire nitrogen cycle of a phytoplankton community it became evident that a nearshore marine environment with convenient access to laboratory facilities was needed. Auke Bay, located near Juneau, Alaska proved ideal for the study. The Institute of Marine Science laboratory facility at Douglas was within easy driving distance and at Auke Bay a small boat wharf was available for docking the M/V MAYBESO, the research vessel used in this study.

In addition to excellent logistical capabilities, the Bureau of Commercial Fisheries Auke Bay Biological Laboratory had accumulated several years of physical and chemical oceanographic data related to Auke Bay and surrounding environs which gave a good background concerning the year-round nature of Auke Bay.

Auke Bay is approximately 2.5 km wide along an east-west axis and 4 km long (Figure 1). The tidal range is from 4-7 m and the wide and deep entrance into Stephen's Passage allows unobstructed tidal motion.

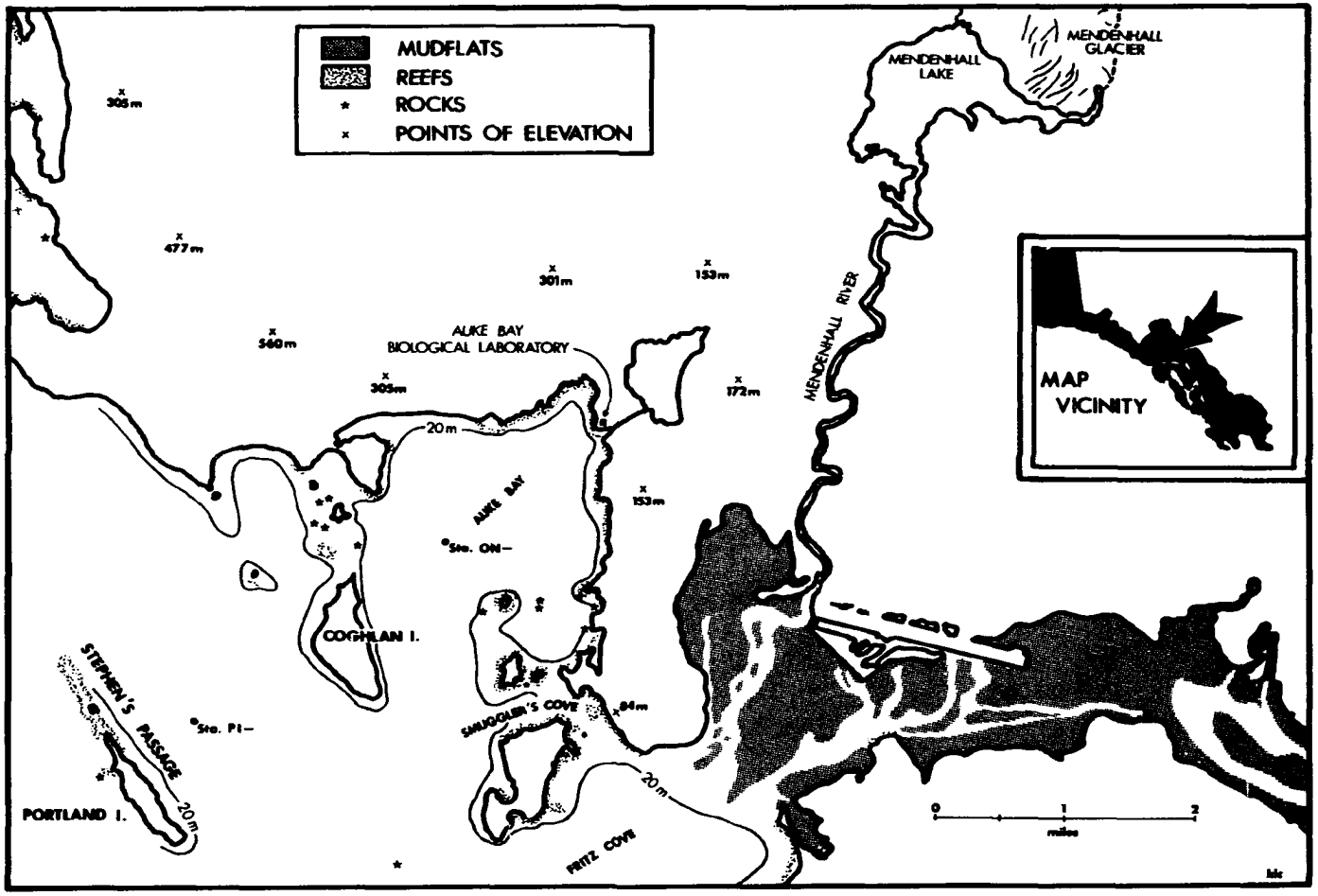

Figure 1. Auke Bay, Alaska. Intensive studies on dissolved organic nitrogen were made at station ON with occasional comparative data from station PI.

Tidal flushing, however, is quite slow and observations by staff of the Auke Bay Biological Laboratory of ice cakes formed during midwinter indicate that the tendency for surface waters to be flushed from the bay was slight (Bruce, personal communication). The bay has a reasonably smooth bottom rising quite suddenly toward the shore. Depth at the middle of the bay is approximately 55 m deepening gradually toward Stephen's Passage to over 100 m just south of Coghlan Island.

In spite of being relatively protected on all sides except the Southwest by heavily forested hills, the severe winds that commonly occur in this region during winter and spring contribute to deep vertical mixing. Nevertheless, the sampling schedule during Spring 1968 was not interrupted because of weather although on two occasions abbreviated stations were taken due to severe winds.

To evaluate as diversified conditions as possible regarding the phytoplankton populations of Auke Bay and the consequent dissolved organic nitrogen, observations were begun in late winter prior to the onset of the spring bloom of phytoplankton. Continuous nutrient monitoring, ^{14}C uptake experiments, chlorophyll-*a* and ^{15}N uptake experiments were conducted during the following period of high productivity and on into late May and early June when nutrient limitations in the surface waters were becoming pronounced. At this time, interference from a large influx of silt-laden water from glacial outflows interrupted the prevailing surface regime and the sampling program was discontinued. For simplicity and ease in comparison of data, all observations were carried out at one location in Auke Bay as indicated

in Figure 1. Occasional comparative work was performed in Stephen's Passage outside of Auke Bay (Station PI).

b. Analytical Background

The processes of identification and quantitative determination of both collective groups of organic compounds and/or individual species of dissolved organic compounds in seawater have been typified by a slow and tedious evolution of analytical procedures capable of detecting the normally minute quantities present. Typical dissolved organic carbon concentrations in seawater are a few parts per million and dissolved organic nitrogen levels may be one-tenth of this amount. When the analytical procedure is then complicated by the presence of some 30 grams/liter of inorganic salts in solution, which often interfere, the problems inherent become formidable. Unless methods can be devised to determine the desired species without sample concentration, the problems of analysis are multiplied by the necessity of extracting the organic compound with immiscible solvents or by precipitating it alone or with carriers. The specter of contamination and handling loss then becomes of great concern to the analyst as well as the problems of incomplete extraction.

Analyses for total dissolved organic nitrogen in seawater have been made for at least 40 years. Krogh and Keys (1934) employed a high temperature combustion method that converted the dissolved organic nitrogen in a 5 ml sample of seawater to ammonia. The ammonia was swept into a solution of HBr and its quantity determined by titration. The sensitivity was marginal for the normal concentrations of dissolved organic nitrogen and involved painstaking care in processing.

Further investigations of dissolved organic nitrogen in seawater were performed by Von Brand and Rakestraw (1941). They used the method

of Krogh and Keys (1934) and described its merit. Although they obtained results reproducible in the laboratory to ±50 µg N/liter, they admitted that at best the analysis was difficult and even an experienced technician could not perform analyses of consistent quality. They recommended that the sample storage be kept to a minimum and recognized the problem of bacterial respiration of the organic nitrogen which released ammonia.

The analytical difficulties precluded further investigation of the dissolved organic nitrogen in seawater until Duursma (1960) conducted an extensive study. By modifying the hydrogenation apparatus of Krogh and Keys (1934), he improved the accuracy and reliability of the procedure somewhat over the previous methods. In addition, the ammonia produced was measured colorimetrically using the sodium phenate method of Riley (1953), which also increased the overall sensitivity. Duursma (1960) painstakingly analyzed samples from various regions of the Atlantic Ocean, reporting levels of dissolved organic nitrogen ranging from <0.04 to 0.40 mg N/kg seawater. Deep water concentrations ranged from 0.10 to 0.20 mg N/kg (approx. 7 to 14 µg-atoms N/liter).

An increased effort toward better analytical methods for isolating and identifying the dissolved organic material in seawater led to the assessment by Jeffrey and Hood (1958) of various means of isolation. They employed procedures using 1) electrodialysis, 2) adsorption on charcoal, alumina, bentonite, etc., 3) ion exchange desalinization, 4) solvent extraction, and 5) co-precipitation of dissolved organic

compounds with ferric hydroxide. The adsorption of organics onto charcoal columns was promising but involved difficulties in subsequent elution. The most successful method for isolating the dissolved organic nitrogen proved to be co-precipitation with ferric hydroxide and this work was followed by investigations describing the dissolved amino acids present in surface sea water (Tatsumoto, et al., 1961) and in deep sea waters (Park, et al., 1962). Eighteen amino acids were identified in the hydrolysates of surface water dissolved organic nitrogen and seventeen amino acids were found in the deep water samples. Threonine, glutamic acid, aspartic acid, glycine and lysine were the most abundant of the amino acids and glucosamine, a monomer of many marine mucilages, was also detected.

Extraction methods were employed by Degens, et al. (1963) to isolate amino acids and other dissolved organic compounds from offshore California seawater. A seawater sample was evaporated to dryness at low temperature and reduced pressure and the residual salt extracted with ethanol. After removing traces of salt by ion-exchange chromatography, the sample was subjected to thin layer chromatography for identification of the nitrogen containing compounds. A variation of the extraction method was employed by Palmork (1963) who converted the dissolved free amino acids to their 2, 4 dinitrofluorobenzene derivatives and then extracted the derivatives from sea water with diethyl ether. Subsequent evaporation of the ether and desalinization by ion-exchange gave a sample which was then subjected to circular paper chromatography. Chau and Riley (1966) re-evaluated the procedures employed by Tatsumoto, et al. (1961), Degens et al. (1964) and Palmork

(1963a) and because of poor and often variable recoveries obtained in each of the above methods, they used a further modification of the direct extraction method of Palmork (1963b). This procedure concentrates seawater by vacuum distillation. The precipitated salts are removed from time to time until the remaining liquid phase is minimal, and then the solution is desalted by ion-exchange chromatography. The eluate, containing the dissolved free amino acids was then subjected to thin-layer chromatography for identification and semi-quantitation through their ninhydrin derivatives. The authors claim their extraction procedure yields about 90% of the dissolved free amino acids in seawater.

The method of Chau and Riley, (1966) although having the advantage of nearly quantitative isolation of the free amino acids, was tedious and subject to several sources of inadvertent contamination as well as also subjecting the dissolved organic nitrogen to the possible deleterious effects of extremely concentrated salt solutions. The possibility of protein denaturation and the release of complexed amino acids into the dissolved free amino acid fraction is readily apparent. Thus this method for the isolation of dissolved free amino acids was superceded almost immediately by the method of Siegel and Degens (1966). By passing seawater through a column of copper-saturated chelating resin, ligand formation between the dissolved trivalent organic nitrogen and the copper selectively removed those compounds containing nitrogen from solution. Subsequent elution of the dissolved organic nitrogen from the column was accomplished with dilute aqueous ammonia. Evaporation of the excess water carried off the ammonia and if it were necessary to totally remove it, the addition of formic acid and gentle heating would sublime the

remaining ammonia away as the formate leaving the dissolved organic nitrogen. This procedure simplified the isolation of the dissolved free amino acid fraction of the dissolved organic nitrogen so that samples could be easily handled on shipboard and a statistically significant quantity of isolates could be obtained with reasonable effort.

Complications involving the use of the copper-chelating resin soon became apparent. The columns tended to become quickly saturated with "humic acids" present in nearshore waters and some of the acidic amino acids were only partially extracted. Webb and Wood (1966) investigated these problems in detail and made modifications in the procedure which improved the efficiency of extraction and reported on the levels of dissolved free amino acids present in York River Estuary, Virginia in July 1965 to January 1966. The overall amino acid nitrogen ranged from a high of 0.8 μg-atom N/liter in July to a low of 0.2 μg-atom N/liter in September. Glycine was the most abundant dissolved free amino acid and accounted for about 20% of the total amino acid nitrogen present. Aspartic acid, glutamic acid, ornithine and serine were also major components of the free amino acids identified.

The copper-chelating resin extraction procedure, coupled with subsequent identification and quantitation of the amino acids isolated by column chromatography on a high pressure ion-exchange amino acid analyzer, represents the current state-of-the-art for amino acid isolation and identification from seawater. This procedure was essentially employed for the work presented herein.

Concurrent with the development of the above methods for isolating specific dissolved organic nitrogenous compounds from seawater, a

major simplification in the determination of total dissolved organic nitrogen in seawater was described by Armstrong, et al. (1966). The dissolved organic nitrogen in seawater was found to be quantitatively photochemically oxidized to nitrate by intense short-wavelength ultraviolet irradiation of a seawater sample in a quartz tube. Within a few hours, the organic fraction was photochemically combusted to yield the organic nitrogen and organic phosphate as the difference between nitrate and orthophosphate levels prior to, and following, irradiation. Armstrong and Tibbets (1968) reported the results of extensive tests on a wide variety of organic compounds regarding their rates of oxidation and found that without exception, all were oxidized by the UV lamp employed. Urea was anomalously slow but was completely oxidized within 24 hr by a 380 watt UV lamp.

This method gives a ready access to the total dissolved organic nitrogen and was employed for the analyses performed in this study. The procedure is discussed in detail in the section on Methods and Experimental Procedure.

It is beyond the scope of this section to include all of the various investigations into specific nitrogenous organic compounds found in seawater, but some of the studies relevant to this work can be summarized. Belser (1959) conducted a series of bioassays for amino acids in waters off the California coast and found considerable quantities of isoleucine, but glycine only occasionally. The sensitivity of the bioassay with a lower limit of about one mg/liter raises serious doubts as to the validity of these results as glycine has been reported most commonly in seawater and isoleucine only rarely. It is reassuring that Litchfield and Hood

(1966) did not detect threonine in the Gulf of Mexico using similar bioassay techniques as levels as high as one mg/liter for any of the amino acids are difficult to anticipate in light of other studies.

Bioassay techniques have been applied to detection of vitamins and growth factors. Litchfield and Hood (1966), (1965) using a mutant of *Serratia marinorubra*, investigated the naturally occurring levels of biotin, adenine and uracil in Gulf of Mexico. Although adenine and biotin were found in occasional marine samples, uracil was found only in water near Mississippi River outflow. It is of interest that many samples contained unidentified factors that were toxic to the bioassay organism.

Menzel and Spaeth (1962) determined vitamin B_{12} in Sargasso Sea samples using the diatom *Cyclotella nana* as a bioassay organism. The levels detected ranged from 5 to over 80 pg B_{12}/liter and approximately reflected the seasonal changes in productivity. Natarajan and Dugdale (1966) used similar techniques and the marine yeast *Cryptococcus albidus* as the bioassay organism for thiamine in North Pacific Ocean waters. Values ranged from <10 pg/liter to a high of 490 ng/liter. In general, open ocean waters contained less thiamine than nearshore waters, and below 75 m it was usually undetectable.

c. Organic Nutrition

The importance of the dissolved organic nitrogen in the role of phytoplankton nutrition can be viewed as a two faceted system. The organic nitrogen may be assimilated as a general source of nitrogen for protein synthesis within the cell or specific molecular species may be transported into the cell and utilized in the form in which they were absorbed. The utilization of dissolved organic nitrogen as a general source of nitrogen would be processes in which amino acids, amino sugars, urea, etc. were transported into the cell and the nitrogen contained in the molecules converted to ammonia through respiration of the carbon, or incorporated into cellular components through transamination. Specific utilization of intact dissolved organic nitrogen is typified by the utilization of extracellular vitamins whereby the absorbed vitamin contributes negligibly to the gross cell structure but instead supplies a necessary factor for cell growth using inorganic nitrogen sources.

The first serious investigation into the biological role of the dissolved organic substances in seawater was made by Pütter (1909), [see Duursma, 1960]. He attempted to demonstrate that zooplankton obtained a major portion of their nutriment from dissolved organic matter. His views were immediately questioned, but it was not until Krogh's (1931) work that analytical techniques enabled investigation into the questions raised. Krogh felt that direct excretion by phytoplankton was very unlikely and in spite of his investigations, no definitely established views have been arrived at concerning the excretion or assimilation of organics by phytoplankton.

Evidence of biological activity associated with the dissolved organic matter in marine waters was, however, soon described. Harvey (1955) and Ketchum (1939) presented evidence that organic phosphates were directly used by marine organisms. In 1956, Tolbert and Zill reported that glycollic acid was excreted by cultures of *Chlorella* and that during certain conditions, the glycollic acid could be readsorbed into the cells. Fogg (1952) reported the excretion of dissolved organic nitrogen by blue-green algae in culture. Most of the organic nitrogen was released as polypetide and amide-N. Also, significantly, most of the released products occurred during fast growth by young cells, which was contrary to the previous suppositions that most dissolved organic matter was released by old and dying organisms. Senescence in cultures did result in dissolved organic matter being released but the relative amount of nitrogen excretion decreased, as the culture aged.

Stewart (1963) expanded the work on blue-green algae demonstrating that rates of release of dissolved organic nitrogen varied considerably from species to species. *Calothrix* released a large percentage of its fixed nitrogen into the medium, rapidly at first and then decreasing in rate for 14 days whereupon the rate of liberation rose sharply for several more days. The overall release rate was highest at the end of the 24 day experimental period. With *Nostoc*, the initial release rate was high and decreased steadily over a 24-day period. The increase in cell population with time yielded a larger quantity of liberated nitrogen at the end of the experiment although the release rate, expressed as a percentage of the fixed nitrogen, was at its minimum.

The reverse flow of dissolved organic nitrogen, that is the assimilation of dissolved organic nitrogen by marine phytoplankton, has been described only recently. Guillard (1963) studied the uptake of organic nitrogen by several species of marine phytoplankton and, in general, found that amino acids were poor sources of nitrogen. These experiments were performed using laboratory cultures.

Hobbie, et al. (1968) described the amino acid flux in an estuary. By measuring the uptake rates of C^{14} labelled amino acids and ambient levels of amino acids in the water, they ascribed quantitative values to their uptake rates. The uptake rates for amino acids, which they ascribed solely to bacterial growth, varied from < 0.006 µg/liter-hour for arginine to 0.423 µg/liter-hour for glycine. Glycine was also the most abundant of the amino acids found in seawater. In a later paper, Hobbie and Crawford (1969) investigated the amount of amino acid carbon that was respired to that incorporated into the cell. Arginine was respired only to the extent of 8% while aspartic acid was respired to the extent of 60% of the amount assimilated from solution. No attempt was made to determine the fate of the nitrogen in the assimilated amino acid molecule.

Definitive proof that dissolved amino acids could sustain algal growth at the low levels that normally occur in seawater was shown by North and Stevens (1967). Growth was demonstrated and uptake kinetics determined on *Platymonas* cultures given glycine as their only nitrogen source at substrate concentrations as low as 1.4×10^{-6} molar. Test cultures employing antibiotics to prevent bacterial growth demonstrated that the uptake of glycine was not due to associated bacterial popu-

lations. The use of tritium labelled glycine as a substrate followed by autoradiography showed that the radioactivity was associated with *Platymonas* cells.

Actual measurement of the uptake of dissolved organic nitrogen in the marine environment was demonstrated by Dugdale and Goering (1966). They innoculated seawater samples from the tropical Atlantic Ocean and the Arabian Sea with glycine and urea labelled with N^{15} and after several hours incubation the phytoplankton were filtered off and their nitrogen fraction subjected to mass spectrometry. In general the uptake rates of glycine and urea were lower than those of nitrate and ammonia but of the same order of magnitude. In several cases, however, urea was taken up faster than ammonia and in nearly all samples of Atlantic Ocean water, urea uptake was faster than that of nitrate. Unfortunately, the ambient levels of glycine and urea in the water samples were not known and hence the absolute uptake rates could not be determined.

Chapter II

METHODS AND EXPERIMENTAL PROCEDURES

a. Physical Parameters

Routine physical measurements of salinity, temperature and light penetration were made at each station occupied in Auke Bay. Salinity and temperature profiles in the top 30 meters were determined with a Beckman Induction Salinometer. The calibration of the salinometer was checked occasionally against secondary standards prepared by the Institute's salinity laboratory. Additional salinity and temperature data was obtained on R/V ACONA cruises 036, 044, and 056. Salinity was determined with an inductive salinometer on samples returned to the laboratory and temperatures by reversing thermometers.

Light penetration was recorded at Auke Bay stations with a Hydro-Products submersible photometer. The extreme variability in daylight in the Auke Bay area coupled with the high attenuation of light during the spring bloom made accurate determination of light penetration difficult. Although a reference cell is normally used to compensate for changes in cloud cover, angle of incidence, etc., one was not available for this study and minor errors were probably made in some light measurements. Fortunately, for the purposes of the experimental program the exact depth of light penetration was not critical.

b. Nutrient Chemistry

Routine determinations of nutrient concentrations in Auke Bay water were made on samples from each station. Water samples were taken with a 10 liter plastic and Teflon sampler, and after collection, 500 ml was filtered through Whatman glass fiber ultrafilters with gentle (10 inches vacuum) suction. All water samples were analyzed for nutrients immediately upon returning to the Douglas Station, usually within two to five hours after being taken. Inorganic phosphate and silicate were determined using the standard methods of Strickland and Parsons (1968). The ammonia method presented a problem as it was desired to use as selective a method for ammonia as possible without becoming too tedious. The standard method developed by Richards and Kletsch (1964) and incorporated into A Practical Handbook of Seawater Analysis by Strickland and Parsons (1968) is non-selective and oxidizes amino acids as well as ammonia. Therefore the method of Grasshoff (1964) which employs a hypobromite oxidation was tested on solutions containing amino acids and found to oxidize approximately 10 percent of the amount present to nitrite. Glycine, however, was oxidized to the extent of forty percent and several attempts to lessen the amount converted to bromamines failed. As a result the ammonia determinations were corrected by the appropriate amount when the glycine content of the seawater was known. For samples in which the glycine concentration was not determined, the correction factor was interpolated from known samples taken as close as possible with respect to depth and time to the unknown sample. This method gave consistent data for ammonia and discrepancies due to amino

acid interference are probably well within the analytical limits of the method. The values for ammonia ranged from 0.5 µg-atoms NH_3-N/liter at the start of the sampling period to near 5.0 µg-atoms NH_3-N/liter in late May and early June. Corresponding values for glycine ranged from 0.1 µmole/liter to a maximum of 1.0 µmole/liter with the average value about 0.2 µmole/liter. If, as assumed, the conversion of glycine to ammonia by this analytical method was fairly constant at about forty percent, the corrections should be reasonable.

The determination of nitrate and nitrite was the most critical of the chemical methods, not only because nitrate was the most important source of nitrogen to Auke Bay phytoplankton, but also because the concentrations of dissolved organic nitrogen were determined indirectly as nitrate as explained below. In essence, the method employed for analysis was an automated version of the mercury - cadmium amalgam reduction method of Morris and Riley (1963) and Grasshoff (1964) although several modifications were made to adapt the method to the Technicon Autoanalyzer system. The column wash was found to operate better at a lower pH so the addition of the NH_4OH to the wash as directed in the manual procedure was omitted. Similarly, natural variations in salinity were found to have an adverse effect on the reduction efficiency of the column and the alternation of saline and wash solution through the flow cell of the colorimeter gave erratic results because of density gradients. Therefore the Autoanalyzer manifold was constructed to dilute the sample with a ratio of 5:1 to 10:1 with distilled water depending upon the nitrate concentration in the sample. The greatly decreased salinity eliminated the deterioration of the

reducing column and the resulting change in density to approximately that of the wash solution allowed smooth flow through the colorimeter. The manifold design is shown in Figure 2. The precision in the 35 µg-atoms NO_3^--N/liter range is ± 0.5 µg-atom N/liter; at the 5 µg-atoms NO_3^--N/liter ± 0.05 µg-atom N/l. The efficiency of reduction was checked periodically against nitrite standard solutions of equal molarity. Optimum reduction of nitrate by the cadmium column gave the equivalent of 95-97 percent of the nitrate as nitrite. After several hundred samples the column would undergo slow deterioration and channeling, resulting in the recorder peaks becoming slightly spread. This condition was easily remedied by the addition of some more 60-80 mesh amalgamated cadmium filings followed by slight compression of the filings into the column. After a few samples had been run through the column, the system would stabilize and remain operative for several hundred more samples.

In the case of unfiltered seawater samples, it was found that a small wad of pyrex wool packed onto the top of the cadmium filings would serve as an effective filter. After one or two hundred samples, the wool would become plugged with detritus and required changing. As expected, this problem was much more evident in highly productive waters.

All nitrate determinations were run in duplicate at a rate of 20 per hour with a blank of distilled water and four standards interspersed between every 36 samples. Thus any contamination or deterioration of the cadmium column was readily detected. No correction for nitrite was made on any nitrate samples run, the two species being reported as nitrate. A preliminary investigation of nitrite levels in Auke Bay and the surrounding area showed that nitrite rarely exceeded 0.3 µg-

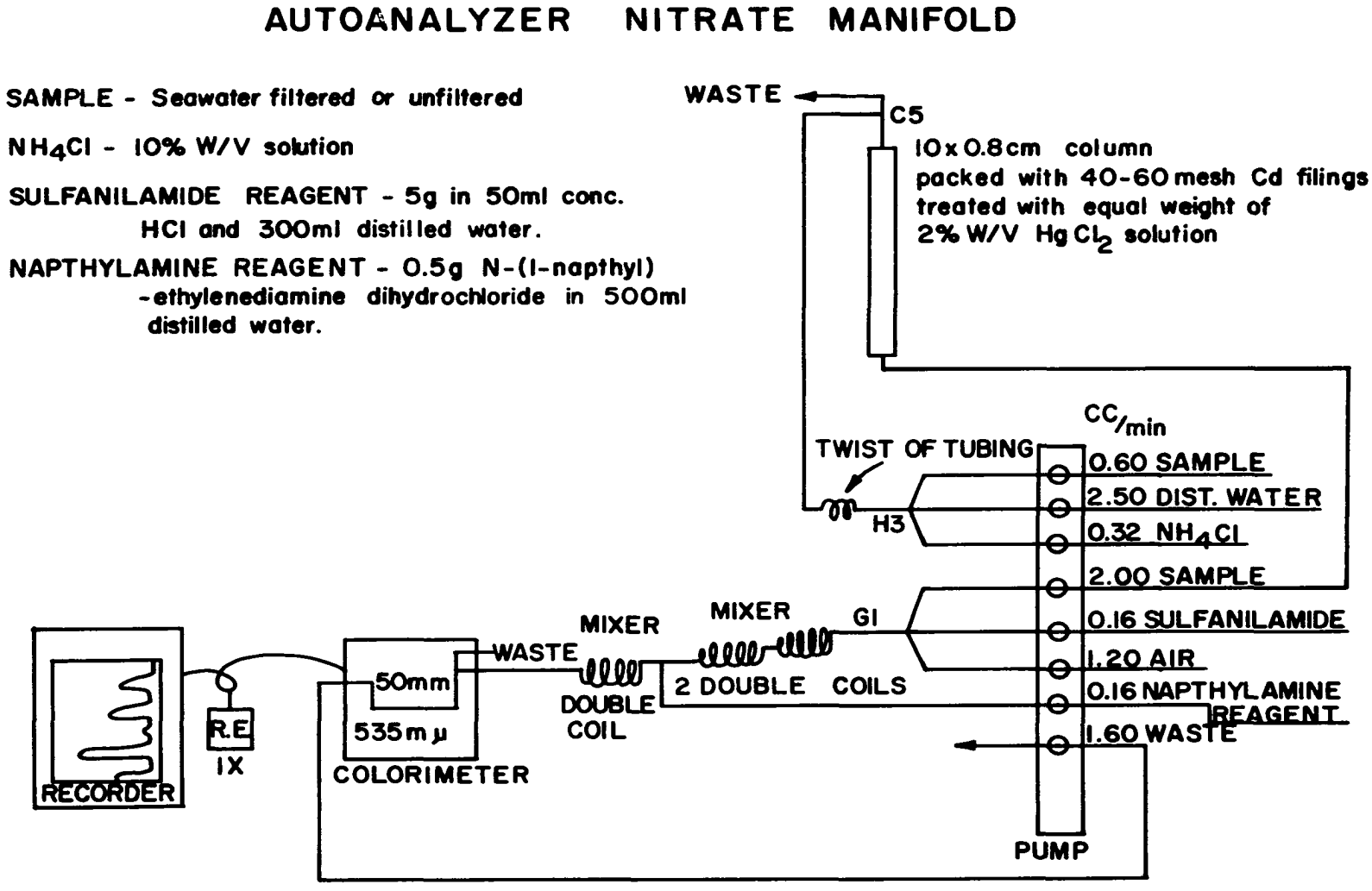

Figure 2. Nitrate-nitrite analysis methodology. For nitrite analysis, the reducing column is omitted and the filtered sample entered on the 2.00 cc/min sample intake.

atom NO_2^--N/liter and in most surface waters was undetectable. Therefore for the purpose of this study the assumption that all of the oxidized nitrogen is present as nitrate is probably valid. If ammonia oxidizing bacteria were present in large numbers, it would not be unreasonable to expect increased nitrite production beneath or at the thermocline during the summer months. For a year-round study in Auke Bay knowledge of the nitrite concentrations would be important; however, this study terminated before any secondary production of nitrate became evident. Nevertheless, the high productivity of Auke Bay would indicate its being a likely site for further studies in nitrification.

c. Dissolved Organic Nitrogen

The excellent reliability and accuracy of the Autoanalyzer nitrate methodology nicely complemented the procedure for the determination of total dissolved organic nitrogen. For the purpose of this study, dissolved organic nitrogen was defined as that organic nitrogen which passed through a Whatman type C glass ultrafilter under gentle suction (10 inches vacuum).

The samples were not treated with any preservative following filtration but were subjected to ultraviolet photochemical oxidation within a few hours of collection. The ultraviolet irradiator consisted of a 30 cm 1200 watt Englehard-Hanovia high pressure mercury arc lamp surrounded by a quartz jacket equipped for cooling with an air stream. The lamp plus jacket was mounted vertically in a lamp shield fabricated from sheet metal and equipped with supports 7 cm from the center of the lamp for 12 of the 125 ml quartz sample tubes. Each of the sample tubes was capped with a 30 ml glass beaker to retard evaporation. The whole irradiator was cooled by a fan which forced air up around the tubes in sufficient volume to prevent the contents of the tubes from warming above 70°C (Figure 3).

Into each of two 125 ml quartz test tubes 100 ml of sample was placed and one drop of 30% hydrogen peroxide. The sample was stirred with a clean glass rod and the tubes placed into the irradiation apparatus. The samples were irradiated for fours hours after which the lamp was turned off and the unit cooled. Each sample was then treated with one more drop of 30% H_2O_2 and stirred. The irradiation was then

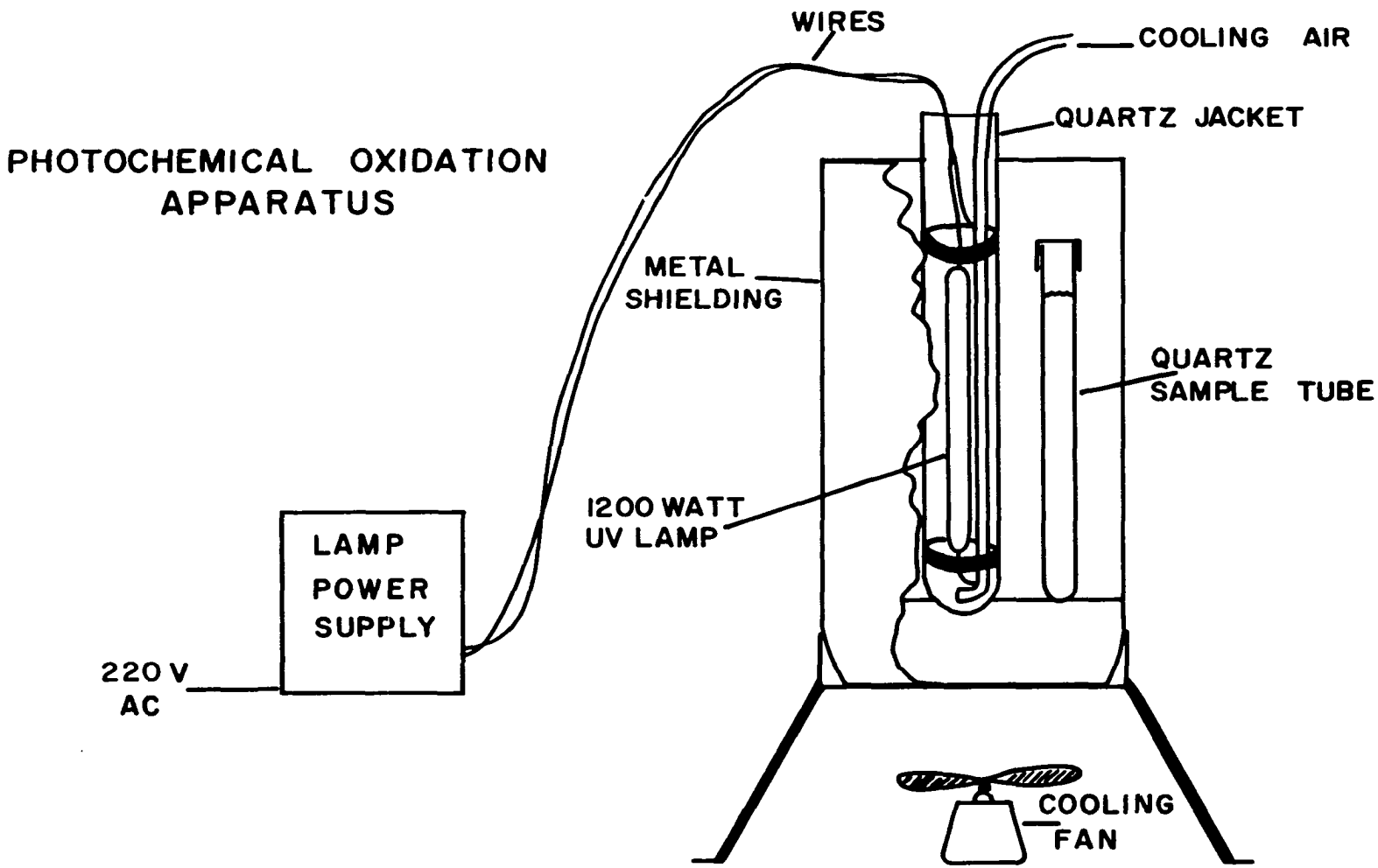

Figure 3. Photochemical oxidation apparatus for the oxidation of dissolved organic nitrogen to nitrate.

continued for two more hours. The time required for the complete photochemical oxidation of the nitrogenous organic fraction was found to be about 4.5 to 5.0 hours with this particular lamp.

Internal standards of glycine, taurine, leucine and glucosamine were oxidized completely within two hours, but the natural organic nitrogen found in seawater took considerably longer. This agrees with the findings of Armstrong and Tibbits (1968), who also noticed the apparent difference in oxidation rates between nitrogenous compounds of low molecular weight and the natural constituents of seawater. Using a low wattage lamp they found that 12 hours of irradiation was required for complete oxidation of naturally occurring organic nitrogen. Of the standard compounds that they investigated, urea was singularly slow in oxidizing to nitrate. Complete oxidation required 24 hours with a 380 watt lamp. The reason for the anomalous behaviour of urea is unknown and they reported that chemically similar compounds oxidized much faster. Transparency to ultraviolet light in the region 200-240 nanometers is not the entire answer because the ammonium ion is oxidized rapidly in spite of being very transparent in the UV.

To empirically evaluate the oxidation rate of the lamp used in this study, Auke Bay seawater was oxidized alone and in combination with 10 µmoles/liter of glucosamine and 10 µmoles/liter of leucine. The seawater and the internally standardized seawater were oxidized in the UV apparatus and 10 ml aliquots taken by pipette on two-hour intervals for nitrate analysis.

Although the results given in Figure 4 show that irradiation was continued for a total of 12 hours, the oxidation was essentially complete

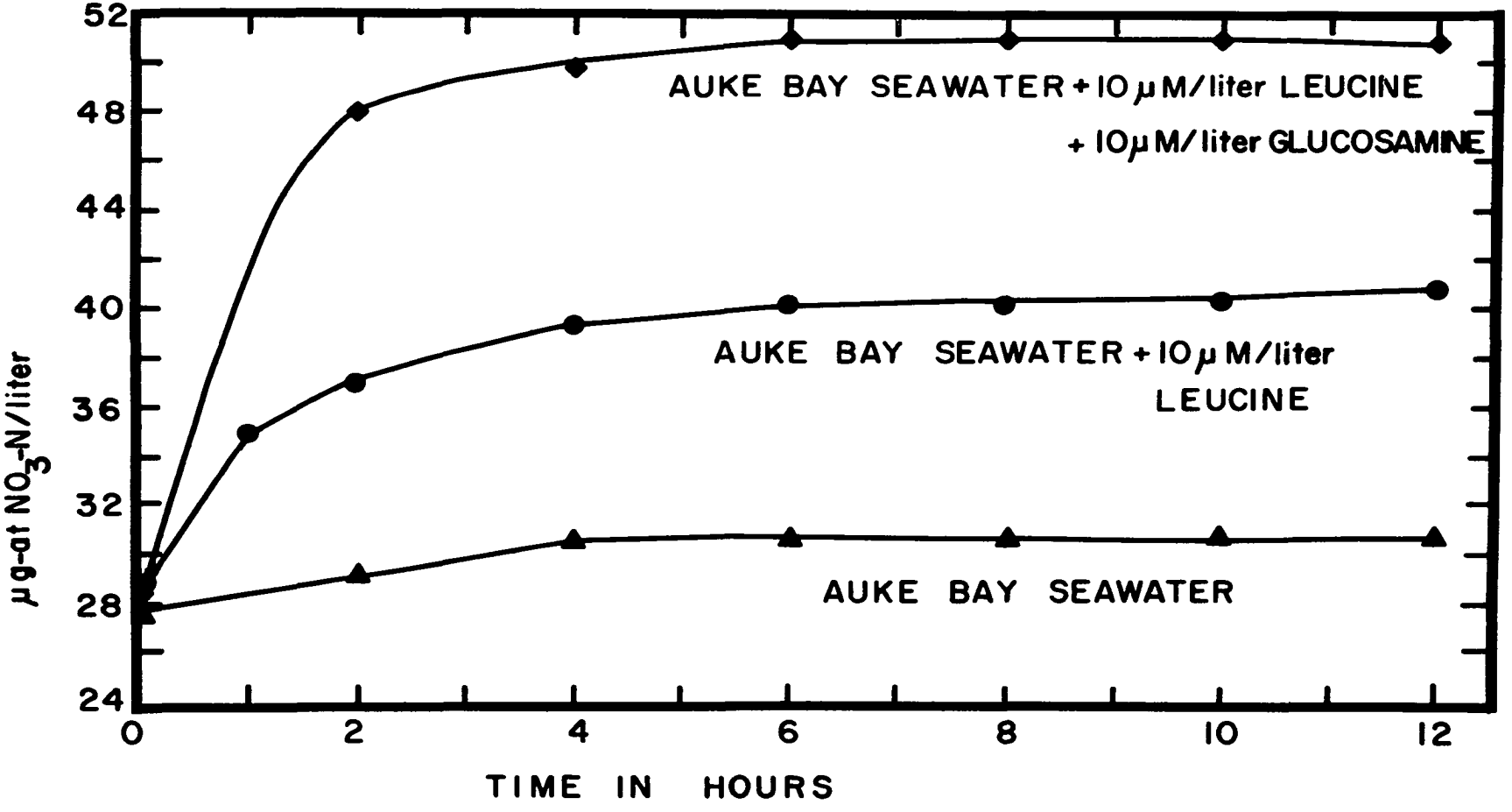

Figure 4. Photochemical oxidation of dissolved organic nitrogen in Auke Bay seawater.

within four hours. However, since the lamp output of the shorter wave lengths of light decreases slightly with continued use, an oxidation time of six hours was used for all the samples run. Occasionally, random samples would be irradiated for an additional length of time to see if the oxidation efficiency of the lamp had decreased appreciably, but this condition was never found.

Upon completion of the oxidation, the contents of each quartz tube were stirred and loaded in duplicate onto the Autoanalyzer sampler tray following the corresponding unoxidized seawater sample. A typical recorder trace of the resulting nitrate levels is shown in Figure 5. The values for total dissolved organic nitrogen in the deeper water presented the most difficulty as the lowest amounts of dissolved organic nitrogen were present here with the highest levels of nitrate. An error in determination of one percent in seawater containing 35 µg-atoms NO_3-N/liter, a value typical of 45 m water, could cause an error in the apparent concentration of dissolved organic nitrogen as high as 10 percent. Thus all samples were run in quadruplicate to provide statistically better values.

Once the phytoplankton bloom had depleted the surface waters of Auke Bay of nitrate, the measurement by difference of dissolved organic nitrogen became much more precise. At a dissolved organic nitrogen level of 5 µg-atoms N/liter, and a nitrate level of 5 µg-atoms N/liter, replicate values for dissolved organic nitrogen did not vary more than ± 0.4 µg-atoms N/liter and averaged ± 0.2 µg-atoms N/liter. This level of precision was fortunately available for most of the experimental work as

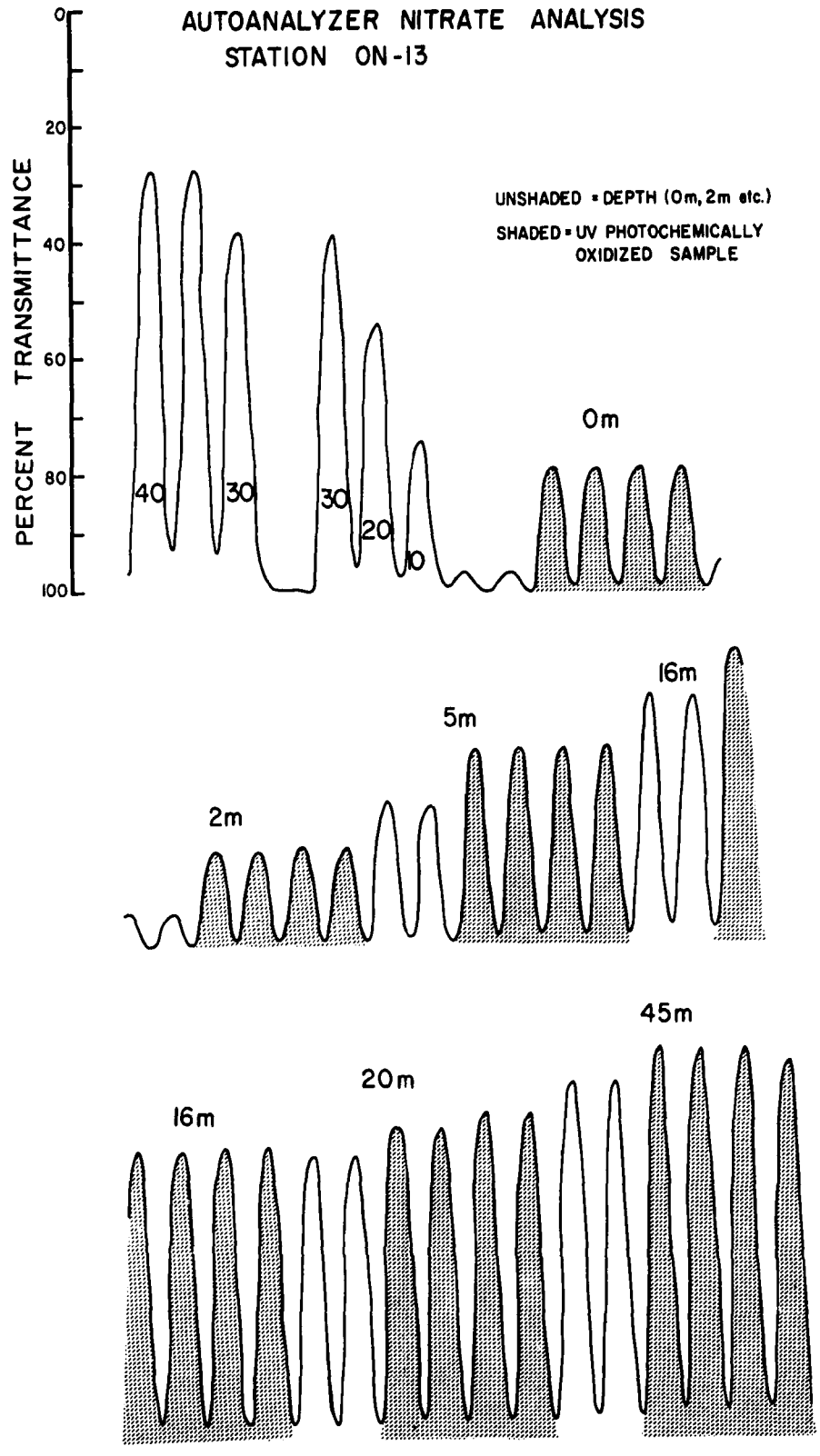

Figure 5. Autoanalyzer recorder trace of station ON-13 nitrate analysis. The unshaded and shaded peaks represent the difference in nitrate levels before and after photochemical combustion of the dissolved organic nitrogen and ammonia. The first six peaks are standards.

nitrate was quickly depleted in the euphotic zone once the bloom had commenced.

d. Amino Acid Isolation and Analysis

The procedure for the isolation and identification of individual amino acids was developed from the method of Siegel and Degens (1964) as modified by Wood and Webb (1966). In the original procedure involving the ligand-exchange chromatography of amino acids on copper-saturated chelating resin, Siegel and Degens (1964) found that micromolar concentrations of amino acids could be quantitatively extracted from artificial seawater and isolated after elution from the resin with dilute aqueous ammonia. The isolated amino acids were then identified and quantitatively measured on an amino acid analyzer. They investigated the concentrations of free amino acids in Buzzards Bay, Massachusetts, and found that the free amino acids in seawater were a small fraction of the total dissolved amino acids. Hydrolysis of the concentrated dissolved organic fraction with hydrochloric acid more than doubled the free amino acid content. They concluded that the large increase in free amino acids upon hydrolysis was not only due to the hydrolysis of peptides but also the hydrolysis of complexes of a phenolic or quinone nature. The presence of hydroxybenzoic acids in the hydrolyzed sample but not in the unhydrolyzed seawater supported this conclusion.

The copper-chelating extraction procedure was extensively tested by Webb and Wood (1966) and problems in the recovery capabilities of the method soon became evident. Standard mixtures of known concentration of amino acids added to seawater were in many cases only partially recovered. Using various internally standardized samples they found that

the efficiency of extraction was increased with increasing pH of the seawater with an upper limit imposed by the formation of inorganic precipitates near pH 9.5. At pH 9.5, they recovered over 90% of most amino acids but the acidic amino acids were only partially recovered, to the extent of about 55% for aspartic acid and 46% for glutamic acid. The results of their recovery experiments are included as Table 1. The abnormally high recovery for glycine was ascribed to a compound that probably co-chromatographed with glycine on the amino acid analyzer used. The experimental procedure described by Webb and Wood (1966) was tried in this study but it was found that upon raising the pH to 9.5, a fine white precipitate was produced and this subsequently quickly plugged the copper-chelating column. Reducing the pH to 9.0 diminished the quantity of precipitate but enough persisted to still present difficulties and a pH of 8.5 was finally used. This reduced the percentage of amino acids recovered and it was therefore decided to adopt a standard dilution technique to accurately evaluate the amount recovered. By adding to the filtered sample a known quantity of norleucine and then performing the chelate extraction, elution, concentration, and amino acid analysis, the amount of norleucine carried through the process served as a reliable indicator of recovery and allowed quantitative estimation of the amino acids present. It is also felt that the use of a lower pH helped to prevent any decomposition of phenolic complexes such as described by Siegel and Degens (1964) which would have given misleading information as to the amount of free amino acids available for phytoplankton assimilation.

Table 1. Percent recovery of 0.25 µmole of amino acids from seawater at various pH's (from Webb and Wood, 1966)

	Percent recovery at pH			
	8.0	8.5	9.0	9.5
aspartic acid	2.9	12.5	28.2	55.0
threonine	13.3	45.9	61.5	85.2
serine	7.1	41.5	63.1	92.2
glutamic acid	1.0	4.2	17.7	46.0
proline	8.4	43.3	52.8	100.3
glycine	23.1	51.7	56.5	115.3
alanine	3.0	17.2	38.7	90.9
valine	3.9	30.2	51.1	90.0
cystine	64.9	53.2	88.0	94.0
methionine	21.6	60.2	81.2	95.7
isoleucine	7.8	38.8	66.6	95.5
leucine	9.3	44.2	70.0	95.3
tyrosine	69.2	84.9	95.4	97.5
phenylalanine	61.1	82.9	94.9	100.7
lysine	2.8	45.8	75.8	95.8
histidine	84.2	82.6	95.4	68.5
arginine	40.6	66.2	84.7	78.9

All glassware that was used in these investigations was scrupulously cleaned to insure against contamination. One misplaced fingerprint contains as much free amino acids as many of the seawater samples and throughout the entire procedure rigorous cleanliness was mandatory. Samples in which serine was a major constituent would have been immediately suspicious as this amino acid is a principal peak in "fingerprint spectra" (Oro and Skewes, 1965). All glassware was first cleaned with detergent and then rinsed with warm nitrous acid, followed by a distilled water rinse. After drying, sample bottles and tubing were wrapped in aluminum foil until immediately before use. With the above cautions and considerations in mind, the procedure for the isolation, identification and quantification of dissolved free amino acids were as follows.

Samples were taken with a lucite and teflon large volume sampler which was externally covered with black polyethylene to prevent light inhibition of photosynthesis by phytoplankton collected at low light depths. From a given depth 2 to 4 liters of water were placed into clean glass aspirator bottles. These samples were immediately filtered under gentle suction with an in-line Millipore filter using 47 mm diameter glass fiber ultrafilters. Filtration was stopped following the collection of four liters and always before the filter sucked dry. The samples were then returned to the laboratory for the amino acid ligand-exchange chromatography.

The pH of each sample was next adjusted to 8.5 using 0.05 N NaOH and an internal standard of 0.5 μmole norleucine was added. The sample

was then allowed to pass at a rate of 6 to 7 ml per minute through a 120 mm x 10 mm glass column filled with 40-60 mesh Biorad Chelex resin which had been saturated with copper. After the entire sample had passed through the column, the resin was rinsed with 50 ml of distilled water and then the amino acids were eluted with 30 ml of approximately 1.5 M NH_3. There was always some shrinkage of the chelex beads during the elution step and the flow rate would be considerably lessened on the next sample. As a result, after use on two or three samples the column would be emptied and refilled with new resin. No attempt was made to recover the resin for re-use.

Following elution the samples were evaporated to near dryness in a 50 ml beaker at 70°C and then two drops of 50% HCOOH were added. At this time there was usually a small quantity of gas evolved, most likely CO_2 from carbonates, and then the sample was again carefully evaporated at 90°C to approximately 0.25 ml. This procedure removed most of the ammonia present. The sample was then taken up with a one ml syringe and placed in a two ml glass ampule. The beaker was rinsed with 0.25 ml of distilled water and the rinse added to the ampule. After sealing the ampule, the samples were frozen at -20°C until ready for analysis.

The amino acid analyses were performed on a Technicon Amino Acid Analyzer using a slightly modified procedure of Hamilton (1963). A 0.3 ml aliquot of each sample was taken from the ampule and run without the addition of other amino acid markers. The eluted amino acids were recorded as peaks on an absorbance scale chart and the locations of the various amino acids were determined by running standard mixtures of protein and nonprotein amino acids. Norleucine was used as a position

calibration marker on the standards and to calculate color yields on the various amino acids with the ninhydrin reagent. To give a continuous check of color yield over the several weeks necessary to analyze all the seawater extracts, standard runs were made after every four samples.

Upon completion of the analyses, the average color yields for each amino acid were calculated and the fraction of norleucine recovered from the original sample ascertained. The concentration of a particular amino acid in the spectrum of a seawater extract was then calculated by the formula:

$$S = \frac{A_s F}{A_a N V}$$

Where: S = amino acid concentration in μmoles/liter

A_s = absorbance of the amino acid from chart

A_a = average absorbance of 0.1 μmole standard of the amino acid

$F = \frac{\text{Percent leucine recovered at pH 8.5}}{\text{Percent amino acid S recovered at pH 8.5}}$

N = fraction of norleucine recovered

V = volume of seawater sample

Average recovery of norleucine in the final analysis was approximately ten percent with an extreme low of 1.1 percent and high of 23.7 percent. In spite of the low recovery, the consistency was good between replicate samples. The concentration of glycine, the most common free amino acid found in Auke Bay, varied an average of 50 percent from the mean in nine pairs of duplicate samples. In the range of 0.05 to 5.0 μmoles/liter which were the concentrations encountered, this was an

acceptable accuracy. Greater accuracy would have required much larger samples and modification of the amino acid analyzer system to the extent of being impractically tedious.

The amino acids that gave peaks equivalent to 0.02 μmole or less on the amino acid analyzer chart were not quantified. The possibility of errors due to slight contamination or systematic problems would indicate a poor validity for attempts to quantitate such low amounts. As indicated earlier, the presence of a relatively high serine peak led to immediate suspicion of contamination. Sufficient duplicate samples were investigated to indicate that serine was a minor component of the naturally occurring amino acids in seawater.

e. Phytoplankton Standing Stock

The particulate nitrogen fraction was measured directly by filtration of a given volume of seawater through a glass ultrafilter. The filter and particulate matter was than combusted in a Coleman Nitrogen Analyzer and the nitrogen gas produced measured volumetrically.

An indication of phytoplankton populations while on station was obtained from Lorenzen's (1966) method of determining *in vivo* chlorophyll-*a*. A sample of seawater was passed through a Turner Fluorometer equipped with a flow cell and the chlorophyll present excited by a high intensity blue lamp filtered with a Corning CS 5-60 filter. The resulting fluorescence was measured through a Corning CS 2-64 filter, and recorded in arbitrary units on a Rustrak recorder. By comparing the particulate nitrogen values obtained from the same sample with the fluorescence, an empirical relationship between fluorescence and particulate nitrogen was established. For fluorescence values ranging from 5 to 25, the ratio of particulate nitrogen/chlorophyll fluorescence was equal to 0.65 with a standard deviation in 12 samples of ±0.07. At values of chlorophyll fluorescence less than 5 or greater than 25, the ratio varied considerably and approximations of particulate nitrogen using chlorophyll fluorescence were unreliable.

f. Tracer Methods: uptake of ^{15}N-labelled substrates

The utilization of ^{15}N as a definitive tracer in the uptake of dissolved organic nitrogen was an easy extension of techniques employed by investigators of nitrogen nutrition in marine waters. Dugdale, Goering and Ryther (1964) used ^{15}N-labelled N_2 gas to show nitrogen fixation by the blue-green algae *Trichodesmium* in tropical waters and with the use of ^{15}N-labelled ammonia and nitrate, the role of fixed inorganic nitrogen was described in relation to primary productivity (Dugdale and Goering, 1967). A preliminary study on the uptake of organic nitrogen using ^{15}N also revealed the ability of phytoplankton to assimilate urea and glycine (Goering, 1966).

The experimental techniques used to measure the uptake rates of ^{15}N-labelled amino acids were very similar to those described by the above authors. In brief, a sample of seawater, usually four liters, was taken from the desired depth and the ^{15}N-labelled amino acid added. For most experiments the ^{15}N was prepared prior to field use as a solution containing 5 µmoles/ml of labelled amino acid. Ampules containing one ml (5 µmoles) of the solution were sealed and sterilized in sufficient quantity to anticipate the experimental needs. After addition of the amino acid to seawater, the samples were stirred and then incubated under the desired conditions chosen for the experiment. Since appreciable changes in the particulate nitrogen fraction due to cell growth or death would affect the uptake rates, incubation times were usually from two to six hours. A reasonably short incubation time also decreased the possibility of the isotopes being assimilated and released

again as an excreted metabolite. After the desired incubation period, the sample was filtered through a glass ultrafilter and the filter rinsed with seawater and dessicated. The sample can be safely stored in this dry state for later isotope analysis. When measuring the uptake of a labelled amino acids, a sample of the same seawater prior to the addition of ^{15}N was taken to determine the ambient concentration of the amino acid in question. Since the concentrations of naturally occurring amino acids were unknown prior to analysis, the addition of 5 µmoles/liter of the acid was chosen to insure that the amino acid would probably be in excess of the maximum amount which could be assimilated during the incubation period. Thus the uptake rates determined by this procedure could be taken as maximum uptake rates for that particular amino acid. To determine the naturally occurring uptake rates, the uptake of amino acids labelled with ^{14}C was employed. The radioisotope technique allowed addition of only minute amounts of amino acids and thereby drastic changes in the ambient concentrations of the acid were avoided. The ^{14}C procedure that was used is discussed later.

To guard against changes in uptake rates caused by a diurnal periodicity, uptake experiments were started at the same time each day, 1000 hours. Ambient light was used for all experiments unless dark conditions were specified in which case the incubation bottles were totally covered with black vinyl tape.

Temperatures during incubation were maintained at that of 2 m seawater by pumping seawater into a plexiglass incubation tank fitted with an overflow drain.

The amount of assimilated nitrogen or carbon that was recycled through respiration could not be determined with the techniques employed in this study. However, a comparison was made of the uptake rates for the ^{15}N-labelled substrates versus the equivalent ^{14}C-labelled substrate to ascertain if the entire molecule or just the carbon or nitrogen of the amino acid was being incorporated. Two one liter flasks of seawater were innoculated with 5 µmoles/liter of ^{15}N-labelled glycine and glutamic acid respectively. Immediately 1 µCi of ^{14}C-labelled glycine was injected into the ^{15}N-labelled glycine sample and 1 µCi of glutamic acid similarly added to the other bottle. Simultaneously, into two four liter glass bottles of seawater, 20 µmoles/liter of ^{15}N-labelled glycine was added to one and 20 µmoles/liter of ^{15}N-glutamic acid to the other.

From the one-liter bottles, a 100 ml aliquot was filtered through 25 mm diameter Millipore HA filters at 2 hrs and at 4 hrs. Similarly, from the ^{15}N-labelled four-liter bottles, two liters were filtered through glass filters at 2 hrs and the remaining two liters from each bottle filtered at 4 hrs. The filters from the one liter bottles were counted for ß-activity as described below and the ^{15}N-labelled filters were analyzed for nitrogen isotope ratios. The uptake rates determined by the two methods were then compared for evidence of respiration or nitrogen excretion.

g. Production of Dissolved Organic Nitrogen

An appreciable rate of assimilation of dissolved organic nitrogen by phytoplankton would soon deplete the levels present in euphotic waters unless either a periodic or sustained production of dissolved organic nitrogen occurred during the spring phytoplankton bloom. To investigate the rate of dissolved organic nitrogen production and in particular the production of dissolved free amino acids, a large-scale incubation experiment was performed. A 100-liter translucent polyethylene drum liner was filled with Auke Bay seawater and suspended from a float in a support of coarse netting at a depth of two meters. After aging for three days, it was emptied and refilled with 2 m seawater, and enough ^{15}N-labelled KNO_3 was added to increase the concentration of nitrate-N from 0.7 µg-atoms/liter to 14.0 µg-atoms/liter. Sufficient KH_2PO_4 (approximately 5 µg-atoms P/liter) was added to prevent phosphate from limiting phytoplankton growth. Immediately after the addition of the labelled nitrate, samples for determining nutrients, chlorophyll, phytoplankton, ^{15}N content of phytoplankton and dissolved free amino acids were taken. Thereafter another set of samples was taken every 24 hours for four consecutive days. The sampling was intended to continue for seven days but a passing boat fouled the anchor line in its propeller and the experiment was terminated. A general implication from the local mariners that such experiments presented a hazard to navigation precluded a repetition of the experiment. Species of the phytoplankton growing in the drum were tentatively identified and rough approximations of their relative abundances were made.

The conversion of the particulate organic nitrogen retained by the glass filters to gaseous nitrogen and the subsequent mass spectrometry procedure have been described by Barsdate and Dugdale (1965). In brief, the glass filters, which have a very small and reproducible nitrogen blank themselves, were ground up with the sample and combusted in a Coleman Nitrogen Analyzer. The nitrogen gas produced was collected in a manifold and bled into a Bendix Time-of-Flight Model 17-210 mass spectrometer and the nitrogen mass peaks recorded. Three replicate spectra were made on each sample and averaged. The sensitivity of the machine was ± 0.01 atom percent for the natural abundance of ^{15}N in the nitrogen of air (0.370 at % ^{15}N).

The nitrogen isotope ratios of the dissolved organic nitrogen in the liquid samples obtained by the Cu-Chelex extraction procedure were determined by loading the sample onto a glass filter and then following the above combustion procedure. The liquid sample, after being evaporated to about 1.0 ml, was treated with two drops of 80% HCOOH and evaporated at 90° C until nearly dry. This removed traces of ammonia left from the extraction procedure. The residue was then dissolved in 0.5 ml distilled water and taken up in a syringe. This solution was then evaporated by placing it drop by drop on a glass filter warmed with a heat lamp. By supporting the filter with a small beaker, the sample could be quantitatively transfered to the filter.

The calculation of uptake rates using nitrogen isotope ratios was performed with standard equations derived for this type of application. The ratios of mass 28 (^{14}N-^{14}N) to mass 29 (^{15}N-^{14}N) were converted to atom percent values with the formula:

$$A_f = \frac{100\ R}{2 + R}$$

where A_f is the final atom percent ^{15}N of nitrogen derived from the filtered particulate material and $R = \frac{\text{mass } 29}{\text{mass } 28}$. The atom percent ^{15}N of the sample is corrected for naturally occurring ^{15}N in particulate material from seawater by subtracting its value from the sample value. This atom percent excess ^{15}N (A_e) is used to compute proportional uptake from the formula, $\frac{A_e}{A_i}$ = proportional uptake, where A_i is the initial atom percent ^{15}N in the experimental substrate. The proportional uptake multiplied by the particulate nitrogen present yields the absolute uptake of the substrate. By dividing the absolute uptake by the time of incubation, the uptake rate of the labelled substrate with time is obtained.

The critical assumptions for the above calculations are that the particulate nitrogen in the system remains constant during the experimental period and that recycling of ^{15}N does not occur. By utilizing short incubation periods, i.e. in the order of hours, these assumptions are probably valid and the uptake rates for the sample substrates investigated are accurate.

h. Tracer Methods: uptake of ^{14}C-labelled substrates

The use of ^{15}N as a tracer in the uptake of nitrogenous organic solutes is subject to criticism in that relatively high concentrations of ^{15}N-labelled substrate are required to give an appreciable increase in the ^{15}N atom percent excess of the particulate matter when used with reasonably short incubation periods. The individual free amino acids present in seawater rarely exceed 0.5 µmoles/liter and thus, the ^{15}N-labelled compound must be added in considerable excess. Therefore to give a reliable indication of the uptake rates of individual dissolved free amino acids at the concentrations occurring in natural waters, ^{14}C-labelled amino acids were employed. Preliminary experiments showed that one microcurie per liter of uniformly labelled amino acid gave good evidence of uptake and this level of activity was used in all experiments. The highest specific activities available, between 95-99% ^{14}C were used to keep the amount of amino acid added minimal. As a result, the total amount of amino acid added ranged from 0.0054 µmole/µCi for phenylalanine to 0.016 µmole/µCi for glycine. These concentrations are less than 10 percent of the naturally occurring levels.

The procedure employed for the ^{14}C-labelled amino acid uptake experiments is as follows: Water from the desired depth was collected with a large volume sampler and placed into four-one liter glass bottles. Black bottles were used where dark conditions were desired. The volume of each bottle was adjusted to one-liter exactly and allowed to equilibrate temperature-wise in an incubator supplied with 2 m water. Then for each sample bottle, a series of 125 ml subsample bottles containing 1.5 ml of formalin were set up. With a syringe, the content

of a one-microcurie ampule of amino acid was carefully injected with rinsings into one of the liter samples. Four such treatments with 4 different amino acids were done. Each sample was then mixed thoroughly and immediately 100 ml of subsample was taken using a fast delivery 100 ml pipette and delivered into one of the 125 ml subsample bottles. The formalin instantly killed the phytoplankton and stopped further amino acid uptake. The first subsample was taken as "zero time" and at one-hour intervals thereafter, 4 additional subsamples were obtained in a similar manner.

In the laboratory, the subsamples were filtered through Millipore 25 mm 0.45 μ filters and rinsed with filtered seawater containing 100 μmoles/liter of mixed non-labelled amino acids. This treatment was used to rinse off any adsorbed labelled amino acids. The filters were then dried at 60°C, placed in planchets and counted for ^{14}C β-activity for ten minutes in a Picker Compact scaler. Background and calibration counts were also made at the start and finish of each experiments' samples.

The ^{14}C counts for each subsample were corrected for background, "zero time", and counter efficiency. The counts/minute were then fitted to a standard regression plot using the least-squares method and the slope used to calculate the uptake rate in micromoles/liter-hr (Young, 1962). This method proved highly sensitive and although it is only an indirect measure of the uptake of organic nitrogen, the results obtained agreed in magnitude with those obtained with ^{15}N.

No correction was made for any ^{14}C respired by the phytoplankton or for the mass difference between ^{14}C and ^{12}C. The loss of ^{14}C through respiration was investigated by comparing apparent uptake rates in the

light and in the dark, and by comparing ^{15}N and ^{14}C uptake rates using the same substrate. If an appreciable amount of ^{14}C was being respired after assimilation, it might be expected that uptake rates in the dark would appear considerably less than values obtained in the light due to photosynthetic assimilation of the $^{14}CO_2$ produced. This did not occur. The consequences of respiration are discussed further below.

Attempts to incubate phytoplankton populations which had the bacterial populations inhibited by the addition of antibiotics failed. A series of four liter bottles of seawater was treated with penicillin-G at 200,000; 100,000; 50,000 and 25,000 units per liter and streptomycin-SO_4 at 50, 25 and 10 mg/liter. Viability of the phytoplankton was checked by the standard ^{14}C productivity procedure after 24 hours of incubation *in situ*. Without exception the diatom populations showed no ^{14}C uptake and were assumed dead and this experimental approach was thus abandoned.

Chapter III

RESULTS AND DISCUSSION

a. Observations on the Physical Environment

The climatic extremes of winter in Southeastern Alaska, typified by strong northerly gales and sub-zero temperatures, thoroughly mix the inland waters to the bottom in the areas studied. Sharma (1969) found that Chatham Strait and Lynn Canal were mixed to below 200 meters by late February. This mixing homogenizes the strong haloclines formed by the heavy summer runoff of fresh water. The fresh water is derived from both the rainfall which ranges from 250 to 500 cm annually, and large quantities of meltwater which descend from the numerous glaciers that originate in the snowfields of the coastal range.

Auke Bay at the start of this study (March 1968) was homogeneous with respect to temperature, salinity and nutrient concentrations from top to bottom. The almost continuous overcast skies and strong winds successfully prevented the formation of a thermocline in spite of gradually warming weather until the first week of April when a very poorly developed one was formed. Development and progression of thermoclinic structure for the spring of 1968 is included in Figure 6. Simultaneously with the first thermally induced water stability the spring bloom began and, since the light initially penetrated to over 20 m, its intensification proceeded about evenly throughout the upper 16 m. The rapid increase in light attenuation by the developing bloom soon prevented growth below 10 m.

Initially the thermocline developed at 6-10 m, but a storm on April 15 completely destroyed it and upon re-forming in late April, water temperatures decreased about uniformly from the surface down to 16 m.

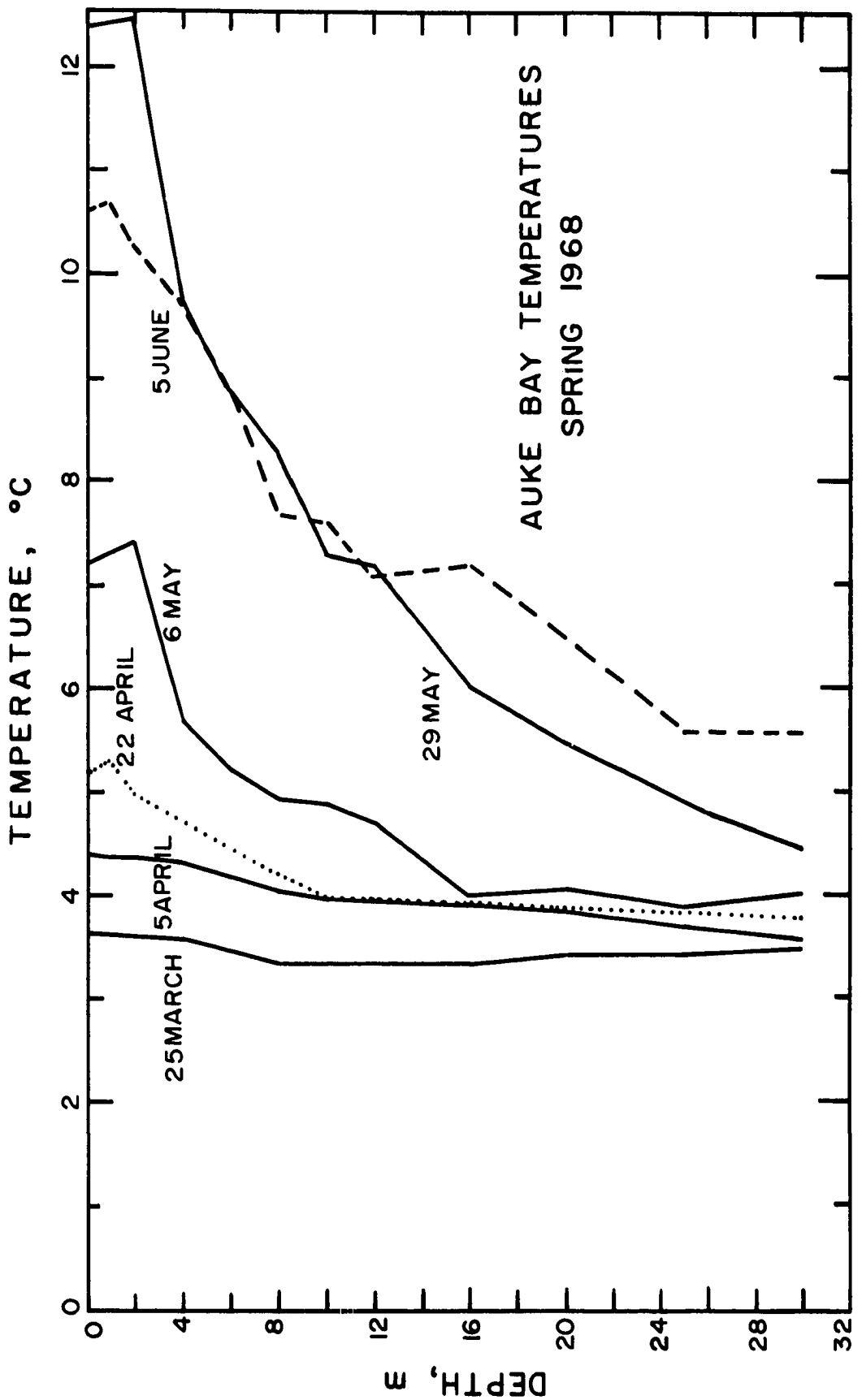

Figure 6. Auke Bay temperature profiles.

The temperature gradient increased as the spring progressed and surface values reached 12°C at the end of May. At this point the rapidly increasing influx of glacial meltwater to Auke Bay interrupted the rising trend of temperature and all observations were discontinued.

Salinity variations were very minor throughout the study period. In late March the surface water exhibited a salinity of 30.0 $^{\circ}/_{\circ\circ}$ which remained unchanged until May 23 when the upper two meters were quite suddenly diluted to approximately 20 $^{\circ}/_{\circ\circ}$ with meltwater.

During the winter and early spring, the Mendenhall River, which originates at Mendenhall Glacier, is little more than a brook as the drainage basin is insignificant in comparison to the great mass of snow and ice in the glacier. The winter temperatures prevent melting and the volume of runoff entering Fritz Cove (Fig. 1) is negligible in relation to the capacity of the wind and tidal flushing to keep the water well mixed. Therefore, the effects of Mendenhall River runoff on salinity and temperature did not become apparent until late May as mentioned above. By late May the volume of the river had increased manyfold and its sediment laden freshwater began spreading over Fritz Cove and into Auke Bay via Smuggler Cove. The glacial flour allowed easy observation of the meltwater on the surface and it also attenuated light penetration to a very shallow depth. The sudden environmental change due to the fresh water influx, served as a sharply defined transition from spring to "summer" conditions. Prior to the appearance of glacial meltwater, the attenuation of incident radiation in Auke Bay was due almost entirely to the diatom blooms. During the spring bloom, the 50 percent light level averaged approximately 3 m and at 10 m less than 1% of incident radiation

remained. The rapid light attenuation coupled with the dark cloudy days that often occurred made light the limiting factor at depths below 3 m during many of the spring days. Conversely, the effect of bright clear days on photosynthesis was dramatically demonstrated on Station 22 (May 16) (see Fig. 9). After several days of low light levels, phytoplankton populations had decreased in the upper 10 meters as nutrients were depleted. A clear period began on May 11 and continued through May 15. The increased penetration and intensity of light caused a rapid phytoplankton bloom to develop at 14-17 m and the particulate nitrogen within that depth interval increased eightfold in 3 days. Similar, but less pronounced variations in photosynthesis, due to fluctuations in light intensity, occurred throughout the spring.

b. Uptake of Inorganic Nutrients

Prior to the spring bloom, the nutrient concentrations throughout the 55 m water column of Auke Bay were uniform. Of the fixed nitrogen available, nitrate-N was the primary source of inorganic nitrogen with a concentration of 31-34 µg-atoms/liter. Ammonia-N was present at less than 1.0 µg-atom/liter. With the beginning of rapid phytoplankton growth, nitrate concentrations rapidly declined as shown in Figures 7 & 8. Ammonia, although a rapidly assimilated nutrient, remained about uniform in concentration during periods of rapid nitrate uptake. This probably resulted from a dynamic equilibrium between ammonia uptake and regeneration. The initial concentrations of dissolved organic nitrogen ranged from 4 to 6 µg-atoms N/liter and this fraction of the available nitrogen, like ammonia, also was probably in a dynamic equilibrium. The equilibria of the amino acid fraction of the dissolved organic nitrogen is discussed in a following section.

Inorganic phosphate was initially present at approximately 2.5 µg-atoms P/liter and if an average N:P ratio of 15:1 is assumed for uptake, the phosphate available exactly complements the amount of available nitrogen. For example, as the bloom progressed the phosphate at 2 m decreased as did nitrate, and as nitrate approached zero on May 1, the phosphate reached a stable minimum of 0.2 µg-atom P/liter. A similar ratio of nitrate and phosphate assimilation occurred throughout the euphotic zone. However, at 16 m phosphate and nitrate uptake occurred only during the initial ten days of the spring bloom (April 1-10) when enough light penetrated to this depth to allow phytoplankton growth. At

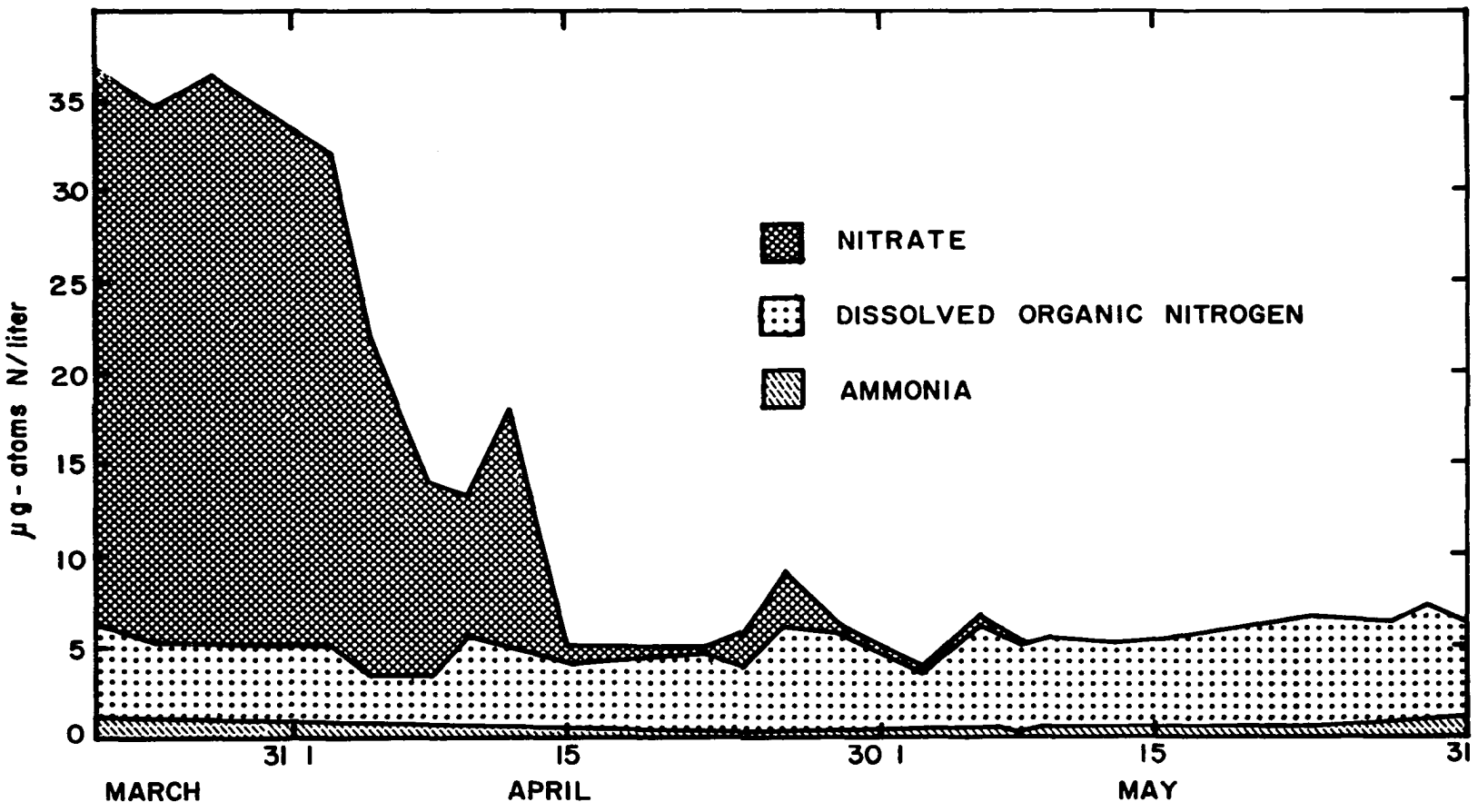

Figure 7. Nitrogenous nutrients in Auke Bay, Om. The Spring phytoplankton bloom began about April 1. The nitrate peaks of April 12 and 27 are due to storm induced mixing.

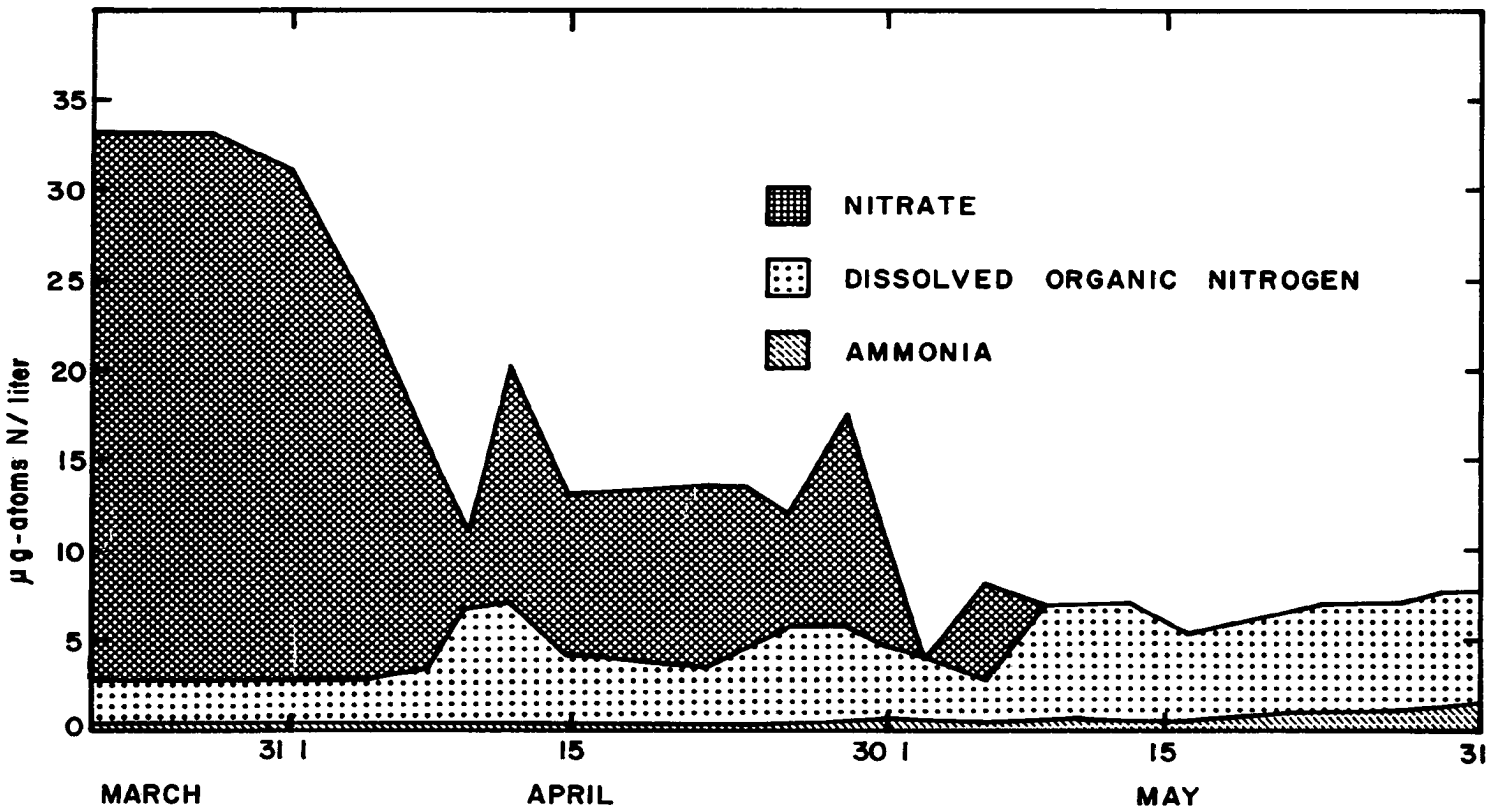

Figure 8. Nitrogenous nutrients in Auke Bay, 5m. Overlay: chlorophyll-a fluorescence and principal diatom species present.

the end of this period, the attenuation of light in the upper layers was sufficient to inhabit further growth at 16 m and no further depletion of nutrients was evident until May 16 when the increased light penetration resulting from grazing and algal deaths in surface waters again triggered diatom blooms. Rapid nutrient depletion accompanied these blooms, but before nutrient levels reached limiting concentrations, light attenuation due to the rapidly increasing particulate matter, again became limiting for growth.

The uptake measurements made during the spring bloom indicated that all forms of fixed N were used continuously. Also the uptake rates do not represent net uptake because corrections for nutrient regeneration are not included. The concentrations of ammonia and dissolved organic nitrogen during the spring bloom varied within narrow limits, and comparison of their ambient concentrations with their uptake rates show that the turnover times for these nitrogen sources are relatively short. The uptake of nitrate is an exception. Its precipitous depletion and lack of any indication of regeneration indicates that the net nitrate uptake/gross uptake ratio is essentially 1.

The rapid nitrate depletion at 5 m which occurred from April 1 through April 10 and again in April 29 to May 3 (Figure 8) averaged 0.11 µg-atom NO_3-N/liter-hr-unit Chl-a and the experimentally measured nitrate uptake rate for these periods, 0.18 µg-atom NO_3-N/liter-hr-unit Chl-a. Unfortunately no information is available as to the diurnal variation of nitrate uptake during the spring diatom blooms. Goering et al (1964) described pronounced cyclic diurnal variations in uptake of nitrate and

ammonia in tropical waters, and because of the energy requirements for nitrate reduction, it is not unreasonable to assume that similar fluctuations occurred here.

Eppley (1970) has noted a diurnal variation in nitrate reductase activity with the activity being lowest at night and highest in late morning hours. If this occurred during this study, then the agreement between the observed natural depletion of nitrate and the measured uptake rates would narrow because the experimental uptakes were made during midday hours, the time of maximum uptake.

Nitrate which is the most abundant source of nitrogen for the spring phytoplankton bloom, is also the most readily utilized form of N during most of the spring. Although ammonia uptake rates were near to those of nitrate, it was not until June, nearly a month after which nitrate had been depleted in the upper 5 m that the uptake rates for ammonia became considerably greater than those for nitrate.

c. Specific Nutrient Concentrations and Their Implications on Species Succession

Relationships between the variety of nutrients available and their respective concentrations, and the succession of the diatom species blooming in Auke Bay were not made obvious in this study. A solution to successions is most likely dependent upon a more detailed definition of the physical and chemical parameters than was obtained. Nevertheless, very pronounced singularities appeared in the diatom populations. Initially, the bloom was composed of almost solely *Rhizosolenia delicatula* and as they declined, secondary populations of *Thalassiosira* sp. followed. By the end of April, 20 days after the peak of the *Rhizosolenia* bloom, no cells of *Rhizosolenia* were evident and the population was mixed consisting of mainly *Thalassiosira* sp. and had an estimated 5-10% each of *Skeletonema* and *Chaetoceros*. Within the next 7 days, May 7 - May 14, the *Thalassiosira* disappeared from the upper 5 m and *Skeletonema* and *Chaetoceros* sp. predominated. At 8 m and below, however, *Thalassiosira* sp. still predominated. The increasing temperature gradient of the upper 10 m (7.25° at the surface, 4.86° at 10 m) may have been responsible for the elimination of *Thalassiosira*.

The overlay for Figure 8 shows the principle diatom species, the chlorophyll maxima, and the corresponding nutrient concentrations at 5 m during the spring bloom. The depletion of nitrate is precipitious during the initial bloom and then remained at low concentrations for about 20 days after which it declined to zero. The three nitrate peaks of April 12, 29, and May 6 were due to turbulent mixing, caused by storm winds,

which brought nitrate up from deeper water. The maximum production of dissolved organic nitrogen occurred during the growth of a bloom population (April 7-9, 22-25) and following that bloom's decline was depleted during a subsequent bloom. Similar results were observed at 0 m and 2 m but earlier depletion of nutrients at these depths and increased mixing may make this interpretation more obvious than accurate as the apparent highs in dissolved organic nitrogen may reflect a decreased rate of uptake with a constant production rate rather than an increased production rate, and a constant uptake rate. The fluctuations do correlate very well with the alternating chlorophyll peaks, suggesting that the changes in diatom populations are a causal factor in changes of dissolved organic nitrogen. The average concentration of dissolved organic nitrogen increased during the spring in the well-lit upper waters of Auke Bay but below 10 m different patterns were noted. The dissolved organic nitrogen fluctuated in response to changing chlorophyll concentrations but conversion to ammonia appeared to be the fate of most of the dissolved organic nitrogen.

Figure 9 shows the relationship between ammonia and dissolved organic nitrogen at 16 m. The intense diatom bloom of *Thalassiosira* sp. on May 13-23 was accompanied by the production of dissolved organic nitrogen. The ammonia was depleted during rapid growth and then appeared to be produced following the bloom at the expense of the dissolved organic nitrogen. This is probably due to heterotrophic consumption of the dissolved organic nitrogen with respiration consuming the carbon and the nitrogen being released as ammonia. It should be noted that nitrate

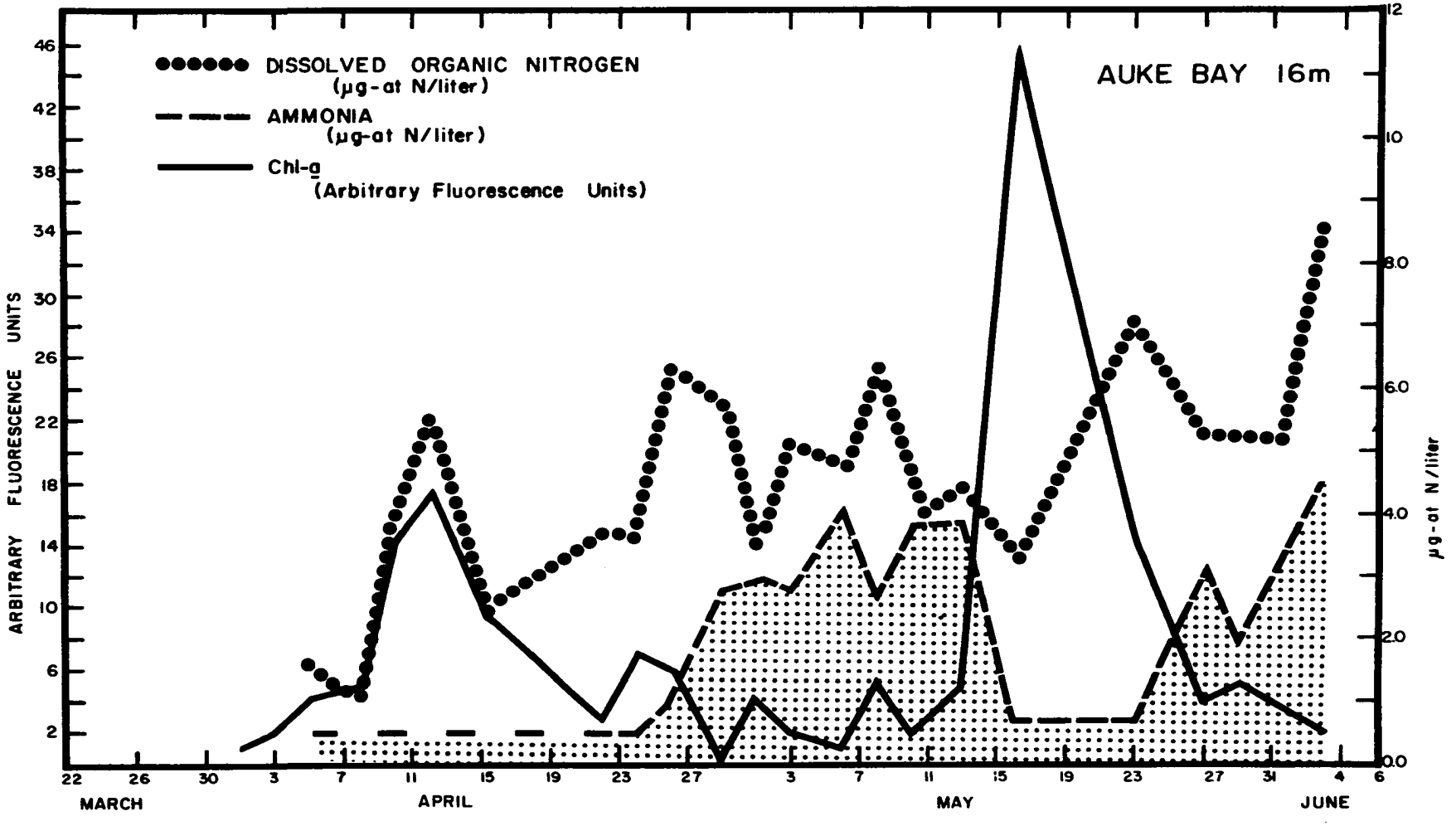

Figure 9. Ammonia, dissolved organic nitrogen and chlorophyll-a in Auke Bay, 16m. The depletion of dissolved organic nitrogen and the appearance of ammonia (April 27 - May 11, May 23 -) is indicative of heterotrophic utilization of dissolved organic nitrogen.

just became limiting at 16 m during the bloom of May 13-23 and that mixing processes partially replenished nitrate soon after. Further evidence of heterotrophic use of dissolved organic nitrogen at 45 m is shown in Figure 10. Chlorophyll was undetectable at this depth until May 1. Then as spring progressed, the dissolved organic nitrogen concentration at 45 m steadily decreased to undetectable concentrations on May 13.

The total reduced nitrogen, however, remained nearly constant over the remaining period of investigation. Chlorophyll measurements indicated that photosynthetic plants were present after May 1 and the concentration of chlorophyll on May 29 was approximately half that of the surface. About 6 µg-atoms particulate nitrogen per liter was associated with this amount of chlorophyll. It is unknown whether the diatoms were sustaining themselves by heterotrophically assimilating the dissolved carbon or if associated bacteria populations were consuming dissolved organic compounds being released by sinking and dying algae. Although temperature measurements were not made below 30 m, the increase in temperature at 45 meters probably did not exceed 1°C during the spring.

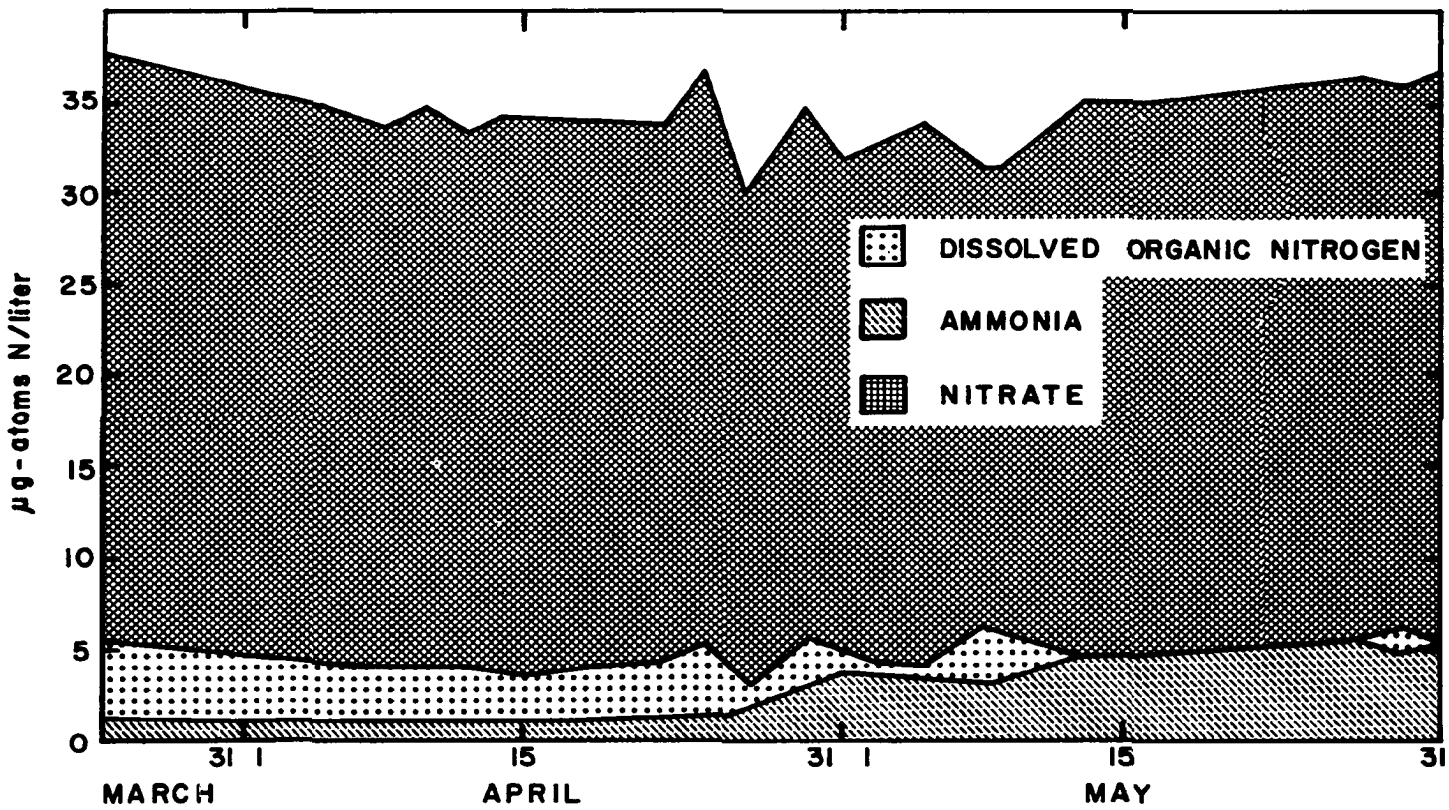

Figure 10. Nitrogenous nutrients in Auke Bay, 45m. The gradual depletion of dissolved organic nitrogen indicates consumption as a carbon source. Chlorophyll-a was detectable at this depth after April 23.

d. Uptake of Labelled Organic Nitrogen

Identification of specific compounds present in the dissolved organic nitrogen was limited to the free amino acids. This limitation, although unfortunately leaving the major fraction of the dissolved organic nitrogen unknown, nevertheless allows some insight into the availability of this nitrogen pool for nutrient purposes. It would be desirable to expand this work to include peptides and other ninhydrin-positive compounds closely related to amino acids. Kittredge (1962) has found, for example, that aminoethylphosphonic acid comprises up to 14 percent of the dry weight of the sea anemone *Anthopleura elegantissima*. In waters containing these or similar species, aminophosphonic acids may be an important excretory product. Similarly, the amino sugars from chitinous residues or other monomers of algal mucus may be present in seawater and may represent significant fractions of the dissolved organic nitrogen pool. Nothing is known about the nature of refractory organic nitrogeneous compounds that may exist and the resistance of the dissolved organic nitrogen found in seawater towards ultraviolet photochemical oxidation certainly indicates that refractory substances are present. The results of the amino acid analyses are tabulated in Table 2. Sixteen amino acids were tentatively identified by their R_f values and one peak remained unknown. This peak often co-chromatographed with glycine and could not be quantitated in many cases. The usual color response was about 10 percent of the glycine peak so when the two eluted together, the total peak was reported as glycine. An attempt to identify the compound by thin layer chromatography was inconclusive because of insuf-

Table 2. Amino acids in Auke Bay seawater, 1968

Station	ON-2	ON-2	ON-6	ON-10	ON-13	ON-15	ON-18	ON-26	ON-27
Date	25 March	25 March	5 Apr.	15 Apr.	29 Apr.	29 Apr.	6 May	31 May	3 June
Depth	2m	50m	2m	2m	2m	2m	0m	2m	45m
glycine	0.02	0.04	0.14	0.5	0.4	0.7	0.7	0.8	0.08
glutamic acid			+	+	0.4	0.3	+		
aspartic acid			+	+	+		+		+
serine		+	+	+	+	+	+		+
threonine			+	+		+	+		+
methionine	+	+			+	+	+		
proline				+					
valine	+	+	+	+	+	+	+	+	
tyrosine					+	+	+		
phenylalanine					+	+	+		
leucine					+	+	+	+	
isoleucine						+	+	+	
histidine						+			
arginine						+			

+ indicates present at < 0.02 µM on analyzer chart

ficient sample. However, upon running a series of nonprotein amino acids and other ninhydrin positive standards on the amino acid analyzer, glucosamine eluted at the position of the unknown compound. Although no further confirming tests were run, glucosamine is often present in marine biochemistry and is probably the unidentified compound. Webb and Wood (1966) using similar analytical techniques also reported an unknown compound in seawater extracts that co-chromatographed with glycine.

Of the free amino acids, glycine was the most abundant, frequently an order of magnitude in excess of the next most plentiful amino acids. Glycine concentrations at 2 m, for example, ranged from the detection limit of 0.02 μmoles/liter to a maximum of 0.9 μmoles/liter during the spring bloom and fluctuated markedly within these limits. Glutamic acid, aspartic acid, valine, methionine and threonine were found in most of the samples but usually at concentrations approaching the limits of detection. The basic amino acids were represented by lysine and histidine in five samples and arginine in two. Concentrations of these compounds were very low in all cases. Serine, as mentioned before, was used as an indicator of possible contamination and was therefore not included in uptake experiments. The remaining amino acids reported occurred sporadically and never in quantities over 0.1 μmole/liter.

Table 3 indicates the relative significance of four amino acids as nitrogen sources to phytoplankton and compares their uptake rates with nitrate and ammonia at an equal particulate nitrogen level. Nitrate and ammonia are between 10 to 50 times as effective as a nitrogen source for phytoplankton as glycine, and in two experiments glycine in turn was four to ten times more readily assimilated than glutamic acid when

Table 3. Uptake rates of organic and inorganic nitrogenous substrates

Station	Date	Substrate	Substrate Conc. µmoles/liter	Uptake Rate µmoles/liter-hr	Uptake Rate per µg-at particulate N_2 (µmoles/liter-hr) x 10^{-2}	Tracer Method
ON-10	15 Apr 68	glycine	0.4	0.01	0.10	^{14}C
PI-1	19 Apr 68	glycine	0.5	0.004	0.23	^{14}C
	"	phenylalanine	0.02	0.00013*	0.005	"
	"	glutamic acid	0.03	0.0004	0.02	"
	"	arginine	0.008	0.00014*	0.005	"
ON-12	22 Apr 68	glycine	5.2	0.016	0.11	^{15}N
	"	glutamic acid	5.0	0.025	0.20	"
	"	ammonia	5.5	0.50	5.0	"
	"	nitrate	5.3	0.50	5.0	"
ON-13	24 Apr 68	glycine	0.4	0.006	0.03	^{14}C
	"	phenylalanine	0.02	0.0003	0.002	"
	"	glutamic acid	0.05	0.0012	0.007	"
	"	arginine	0.008	0.0001*	0.0006	"
ON-17	3 May 68	glycine	5.5	0.028	0.14	^{15}N
	"	glutamic acid	5.0	0.016	0.08	"
	"	ammonia	5.3	0.75	3.7	"
	"	nitrate	5.1	1.00	5.0	"
ON-27	3 June 68	glycine	10.1	0.012	0.21	^{15}N
	"	glutamic acid	10.0	0.017	0.29	"
	"	ammonia	6.3	0.133	2.3	"
	"	nitrate	5.0	0.063	1.1	"

* rate based upon amount of ^{14}C-labelled amino acid added

present at natural concentrations. Glycine and glutamic acid at concentrations of 5 µmoles/liter were assimilated at approximately equal rates, indicating that the differences in uptakes rates at natural concentrations is mostly due to the differences in ambient concentrations. The fact that glycine uptake rates at natural concentrations of 0.4 µmoles/liter are essentially equal to the uptake rates at 5.0 µmoles/liter also indicates that the transport mechanisms for glycine assimilation are nearly saturated at the concentrations normally present in the natural environment. Glutamic acid, conversely, was found to be limited to about 10% of its maximum uptake rate because of the low ambient concentrations. The overwhelming preference for the inorganic forms of nitrogen is impressive even when considering the effects of fairly severe inorganic nutrient limitation. Work by Eppley et al (1968) and MacIsaac and Dugdale (1969) indicates that the half-saturation constant, K_s, for nitrate uptake for neritic diatoms range from 0.5 to 2.0 µg-atom N/liter and for ammonia uptake, 0.5 to 3.0 µg-atoms N/liter. These are the concentrations at which growth occurs at half the maximum possible rate if the nutrient were in excess. The lower figure of 0.5 µg-atom/liter is similar to the concentration of glycine in natural waters and this would represent a saturation nitrate (or ammonia) uptake rate of about ten times that of glycine or glutamic acid. This shouldn't be interpreted as indicating that dissolved amino acids are unimportant in diatom nutrition. Indeed, by April 29, nitrate had been depleted to 0.3 µg-atom/liter at 2 m and by May 9 had reached undetectable concentrations (<0.05 µg-atom/liter). This total lack of nitrate left only ammonia and dissolved organic nitrogen as nitrogen sources and ammonia

at the 2 m level remained at less than 1.0 µg-atom/liter until nearly June 1. If the average K_s for ammonia is near to 1.5 µg-atoms/liter then the combined uptakes of the dissolved free amino acids share nearly equal roles with ammonia in the summer nutrition of phytoplankton.

As an example of the significance of dissolved amino acids as a source of nitrogen for primary production, the contribution of free amino acids to the total nitrogen requirements at station ON-17 can be estimated. Primary productivity not obtained on station ON-17 but at station 18, the closest productivity value available timewise, an average carbon fixation rate of 102 µg C/liter-hr or 8.5 µg-atoms C/liter-hr was obtained. Assuming a C:N:P ratio of 100:15:1, the 8.5 µg-atoms C would require 1.25 µg-atoms N/liter-hr. Of this amount, glycine supplied 0.03 µg-atom N/liter-hr and glutamic acid, 0.06 µg-atom N/liter-hr, totaling 7.5% of the required nitrogen input. Ambient concentrations of glycine and glutamic acid were approximately 0.7 µmoles/liter and 0.02 µmoles/liter respectively. The inorganic nitrogen, nitrate and ammonia, were 0.6 µg-atom NO_3-N/liter and 0.7 µg-atom NH_3-N/liter, which, considering the large K_s values given above, indicates that these concentrations would be severely limiting. The total dissolved organic nitrogen pool was 5.1 µg-atoms N/liter and thus had the potential and probably did supply a large quantity of the required nitrogen.

e. Turnover Times of Dissolved Free Amino Acids

The time required for the uptake of an amount of amino acid equivalent to the amount present in solution at a given time is defined as the turnover time. Turnover times give an indication of the efficiency of the biological transport systems capable of assimilating the individual species of amino acids. By using ^{14}C-labelled amino acids, the uptake of amino acids and their turnover times were conveniently determined on three cruises in Alaskan waters as well as during the Auke Bay study (Table 4).

The values given here for turnover times do not reflect the losses of assimilated carbon through respiration. As a result, the turnover time given may be in error as high as 50%. These values, are used however, to reflect the turnover time with respect to actual incorporation of the amino acid into cells. The problem of respiration is discussed in detail in the following section.

R/V ACONA Cruise 036 samples were taken at station 1044 in Peril Straits, southeastern Alaska. Spring bloom conditions were commencing at this time. Cruise 044 samples were from Prince William Sound and represent nearly sterile summer surface waters and the turnover times obtained on Cruise 056 are typical of winter conditions in southeastern Alaska. Turnover times of several hundred hours occurred in waters nearly devoid of phytoplankton, whereas in general, the shortest turnover times were found in waters with high phytoplankton concentrations. At a given high concentration of chlorophyll there can be extreme variations in the turnover times as evidenced by the uptake data for

Table 4. Turnover Times for Dissolved Free Amino Acids

Amino Acid	Cruise	Station	Location		Date	Turnover Time (hours)	Chl-a fluorescence arbitrary units
phenylalanine	036	1044	57°33'N	135°29'W	5 April 67	65	14
"	044	Redhead	60°37'N	146°34'W	15 June 67	850	0.1
"	Auke Bay	ON-13	58°22'N	134°41'W	24 April 68	61	24.0
"	"	PI-1	58°21'N	134°43'W	19 April 68	60.3	2.0
arginine	036	1044	57°33'N	135°29'W	5 April 67	150	14.0
"	044	Redhead	60°37'N	146°34'W	16 June 67	690	0.1
"	044	Stockdale	60°19'N	147°12'W	15 June 67	505	0.1
"	Auke Bay	PI-1	58°21'N	134°43'W	19 April 68	56.2	2.0
"	"	ON-13	58°22'N	134°41'W	24 April 68	75	24.0
glycine	044	Redhead	60°37'N	146°34'W	16 June 67	680	0.1
"	056	1990	57°17'N	133°57'W	15 November 67	1600	0.1
"	Auke Bay	ON-10	58°22'N	134°41'W	15 April 68	42	11.0
"	"	PI-1	58°21'N	134°43'W	19 April 68	119	2.0
"	"	ON-13	58°22'N	134°41'W	24 April 68	73	24
"	"	ON-16	58°22'N	134°41'W	1 May 68	16	24
glutamic acid	044	Stockdale	60°19'N	147°12'W	15 June 67	425	0.1
"	056	1990	57°17'N	133°57'W	15 November 67	675	0.1
"	Auke Bay	PI-1	58°21'N	134°43'W	19 April 68	69	2.0
"	"	ON-13	58°22'N	134°41'W	24 April 68	43	24
"	"	ON-16	58°22'N	134°41'W	1 May 68	8.8	24

glycine and glutamic acid at stations ON-13 and ON-16. The wide disparity is not due to large amino acid ambient concentration differences, as evidenced by the glycine concentration of 0.3 μmoles/liter at ON-16 and 0.4 μmoles/liter at ON-13, but must instead result from differences in the adaptation of the phytoplankton transport systems to assimilate free amino acids.

f. Respiration and Net vs. Gross Uptake of Dissolved Organic Nitrogen

The fate of a molecule of amino acid or other organic substrate after initial assimilation into the cell can be assumed to be either oxidation through respiratory processes or incorporation of part or all of the molecule into the structure of the cell. To determine the relative amounts of the substrate employed in either respiration or incorporation, Hobbie and Crawford (1969), using ^{14}C-labelled amino acids measured the fraction of ^{14}C respired and incorporated in estuarine and pond waters. They found that the carbon skeletons of the various amino acids varied greatly as to the fraction respired, with the acidic amino acids in general, the most readily oxidized and the basic amino acids almost completely incorporated into the cell. Glutamic and aspartic acid were respired to the extent of 61% and 60%, respectively, whereas, lysine and arginine were respired to the extent of 11% and 8%. The neutral amino acids investigated varied in their susceptibility to respiration over incorporation, with an average respiration of about 28% of the assimilated acid. No attempt was made to investigate the fate of the amino nitrogen.

In this study no attempt was made to determine the relative rates of respiration and incorporation because the primary interest was to assess the net assimilation (i.e. incorporation) of the organic nitrogen into the cell and hence its potential for sustaining growth of phytoplankton populations. However, an experiment was conducted to determine the relative amounts of organic carbon and amino nitrogen that were incorporated into the phytoplankton from a given organic substrate.

Since both ^{15}N and ^{14}C labelled glycine and glutamic acid were available for this study and were the prominent species of free amino acids dissolved in seawater, they were chosen for this investigation.

Four samples of 2 m seawater from station ON-19 were innoculated with 5 μmoles/liter each of ^{15}N labelled glycine and two of these were additionally innoculated with one microcurie of ^{14}C glycine (0.016 μmoles). One bottle from each of these two treatments was incubated in the dark and the remaining pair incubated in the light. A similar set was prepared using ^{15}N and ^{14}C labelled glutamic acid. After incubating for six hours, the samples were filtered and depending on whether the label employed was ^{14}C or ^{15}N, the filters counted or processed for isotope ratios. The uptake rates are given in Table 5.

No estimates can be made of the amount of amino acid lost by respiration since no attempt was made to isolate and count the respired carbon dioxide. The values in Table 5 are measures of the relative incorporation of the amino nitrogen and the carbon skeleton of the amino acids. In all cases the uptake rates were faster in the dark, but this could have resulted from an inhibitory effect on photosynthesis of the bright sunshine during incubation. The ^{14}C of the glutamic acid was incorporated into the cell at a rate nearly 2.5 times faster than ^{15}N and the reverse effect was noted for glycine. This suggests that different biochemical processes are operative in the assimilation of these organic compounds. Without considerable further experimentation one can only speculate on the major pathways of their assimilation. It can be noted that through deamination to α-ketoglutarate, glutamic acid

Table 5. Uptake rates of ^{14}C and ^{15}N labelled glutamic acid and glycine. Values shown are the average of rates determined at 4 hr and 6 hr incubation. Amino acid concentration = 5.0 µmoles/liter

Amino Acid	Conditions	^{14}C Uptake Rate	^{15}N Uptake Rate
glycine	light	0.004 µmoles/liter-hr	0.006 µmoles/liter-hr
glycine	dark	0.007 µmoles/liter-hr	0.011 µmoles/liter-hr
glutamic acid	light	0.010 µmoles/liter-hr	0.004 µmoles/liter-hr
glutamic acid	dark	0.017 µmoles/liter-hr	0.006 µmoles/liter-hr

may be incorporated into the citric acid cycle. Glycine can, in contrast, by addition to succinyl coenzyme A begin the series of reactions leading to porphyrin synthesis which is a synthesis occurring in growing phytoplankton. This mechanism would retain the nitrogen in the cells. Glutamic acid, during formation of α-ketoglutarate loses its nitrogen as ammonia and although it would be available for other pathways of incorporation, it may instead diffuse back into the environment. Biochemically valid arguments can be applied in the opposite direction, that is, for the retention of nitrogen in the case of glutamate and for the loss of glycine nitrogen by transamination or, deamination but such arguments serve no purpose until experimentally verified. The examples given above are only possibilities why there are large differences between the rates of carbon versus nitrogen incorporation of glutamic acid and glycine. The true explanation of this phenomenon must await further experimentation.

g. Production of Dissolved Organic Nitrogen from Nitrate

The utilization of the nitrate anion by phytoplankton and the subsequent rate of release of the nitrogen back into the marine environment was investigated in a single large-volume experiment as described in the section on Methods and Experimental Procedures. The experiment consisted of filling a large polyethylene incubator with seawater into which a known amount of $K^{15}NO_3$ was added. Samples of seawater were taken every day for 4 days for the determination of nitrogen isotope ratios of the nitrogen contained in the particulate and the dissolved organic nitrogen matter.

The initial response to the nitrate enrichment was a burst of phytoplankton growth that raised the total particulate nitrogen fraction from 10.2 µg-atoms N/liter to 28.5 µg-atoms N/liter in two days, simultaneously depleting the entire supply of added nitrate. Within 24 hours after addition of nitrate the dissolved organic nitrogen increased from 3.1 µg-atoms N/liter to 4.3 µg-atoms N/liter and continued to increase for two more days, peaking at 5.8 µg-atoms N/liter at 72 hr. At 96 hr, the dissolved organic nitrogen had declined to 5.2 µg-atoms N/liter. Ammonia levels remained essentially unchanged throughout the experiment.

The free amino acid concentrations in the incubation medium responded almost opposite to the total dissolved organic nitrogen concentration. Glycine and glutamic acid were present initially at concentrations of 0.7 and 0.02 µmoles/liter respectively but within 24 hr glutamic acid was undetectable and glycine declined to 0.1 µmole/liter. At 48 hr

the glycine concentration was 0.08 µmoles/liter and it then rose sharply to 0.7 µmoles/liter at 96 hr. Glutamic acid remained at less than 0.02 µmoles/liter.

The isotopic analysis of the particulate and dissolved organic nitrogen indicated that nitrate-N was quickly cycled into the dissolved free amino acids. Unfortunately the sample of amino acids taken after 24 hr was lost in handling but the 48 hr sample gave an atom-percent ^{15}N excess of 4.20%. The particulate fraction at the same time having an atom percent ^{15}N excess of 43.9% N^{15}. If the ^{15}N is assumed to be uniformly distributed in the particulate nitrogen and the particulate nitrogen is the sole source of the dissolved organic N, this would indicate that approximately 9.5 percent of the dissolved free amino acids and other organic nitrogenous compounds in the Cu-chelex extract were of phytoplankton origin. Since the atom percent ^{15}N in the particulate fraction was increasing with time through nitrate assimilation, this estimate is probably conservative. Also, the experimental difficulties in removing all traces of ammonia introduced during processing these samples requires that quantitation of actual amounts of produced free amino acids be taken with reserve, but this realization also requires that the ^{15}N excesses be regarded as the lowest probable values. Traces of ammonia left during sample preparation would dilute the ^{15}N present.

h. Ecological Implications of Dissolved Organic Nitrogen in Auke Bay Phytoplankton Populations

The role of dissolved organic nitrogen as a direct nutrient for phytoplankton growth in Auke Bay is probably minor during most of the spring bloom when inorganic sources of nitrogen are present at levels over 3-4 µg-atoms N/liter. However, as suggested by Provasoli (1964) various components of the dissolved organic nitrogen may act as important growth enhancement agents through chelation or as essential vitamins.

With the depletion of the inorganic nutrients in the euphotic zone by late spring, the role of dissolved organic nitrogen assumes increased importance. The stabilized water column coupled with the removal of senescent populations through sinking result in a drastic depletion of inorganic nutrients in the uppermost water. At this time, dissolved organic nitrogen is the only sizeable source of fixed nitrogen and offers a source of nitrogen for phytoplankton species that are physiologically adapted to its use. The increased rates of utilization of dissolved organic nitrogen that occurred late in spring support this contention.

The particulate nitrogen that is removed from the surface by sinking is regenerated near the thermocline by bacterial respiration of the carbon and release of ammonia. It is perhaps very significant for phytoplankton growth that the end product of the movement of nitrogen from the particulate to the dissolved fraction at this deeper level (16-20 m) is as ammonia and not dissolved organic nitrogen. The extreme suitability of ammonia over dissolved organic nitrogen for phytoplankton nutrition allows sudden outbursts of growth during short periods (1-3 days) when light penetrates to these depths. Favorable light conditions can result

from periods of bright sunshine or periodic decreases in phytoplankton densities. The dissolved organic nitrogen that is carried to deep water by mixing or currents during summer apparently serves as a carbon source for other microorganisms. The complete conversion of the dissolved organic nitrogen to ammonia that occurs at 45 m in Auke Bay in late spring likewise indicates the biological significance of dissolved organic nitrogen.

No attempt has been made to establish a nitrogen balance for Auke Bay because of the complexities of the biological activity involved in nitrogen transformation. Zooplankton grazing of phytoplankton was slight during most of the early spring bloom and then increased as the number of zooplankton increased rapidly toward the end of May. Also, in late May, large schools of herring entered the bay to spawn followed by salmon and finback whales. The impact of several whales, salmon, hake and other predators on the herring and the consequent impact on zooplankton grazers phytoplankton production, and nitrogen transformation was beyond estimation.

It is prudent to add here that the effects of zooplankton contributions to the dissolved free amino acid pool have been disregarded. Webb and Johannes (1967) reported that zooplankton released dissolved free amino acids. The release rates were temperature dependent and below 6°C no organic nitrogen was released. Shortly following this work Corner and Newell (1967) investigated the excretion products of *Calanus helgolandicus* and found that no free amino acids were excreted by uncrowded animals and that only when the animals were concentrated did ninhydrin positive organic products appear. Ammonia was essentially

the sole nitrogen form excreted. In view of the above work and the low apparent numbers of zooplankton retrieved in plankton tows, it was felt that only minor amounts of dissolved organic nitrogen were produced by zooplankton.

SUMMARY

The difficulties involved in previously available analytical procedures for determination of dissolved organic nitrogen in seawater have prevented detailed investigation of this fraction of the marine nitrogen pool. Recent analytical developments, in particular the photochemical combustion of dissolved organic nitrogen to nitrate with high intensity ultraviolet light and the extraction of amino-nitrogen containing compounds from seawater with copper-saturated chelating resin, have allowed expansion of the investigations concerning dissolved organic nitrogen in phytoplankton nutrition. This study has assessed the importance of dissolved organic nitrogen in relation to nitrate and ammonia as nitrogen sources for phytoplankton growth during the spring bloom in Auke Bay, Alaska. Several conclusions resulted from this investigation.

1) The concentration of dissolved organic nitrogen in Auke Bay was uniform at 3-4 µg-atom N/liter throughout the water column following winter mixing. Following thermocline formation and the onset of the spring bloom, dissolved organic nitrogen concentrations fluctuated from <1 to 8 µg-atoms N/liter in the euphotic zone. Changes in phytoplankton densities occurred with changing concentrations of dissolved organic nitrogen although a clear cause-and-effect relationship could not be established.

2) The dissolved free amino acids present in seawater are utilized and excreted by phytoplankton. Glycine and glutamic acid were the most abundant amino acids isolated, ranging from <0.1 to 1.0 µmole/liter

over the period of the spring bloom. Fourteen other ninhydrin positive compounds were identified in seawater extracts but concentrations were usually less than 0.05 µmole/liter. The uptake rates of amino acids by phytoplankton are much slower than those of nitrate and ammonia when the latter sources of nitrogen are in abundance. Nitrate and ammonia were each utilized nearly 30 times faster (0.05 µg-atom N/liter-hr x µg-atom particulate N) than glycine or glutamic acid when the inorganic nutrients were in excess. Upon depletion of nitrate and ammonia, in the euphotic zone, the ratio of their combined uptake rates to glycine or glutamic acid decreased to 7. The total quantity of dissolved organic nitrogen was probably contributing the major portion of nitrogen required for phytoplankton growth in periods of nitrate depletion. At station ON-17, glycine and glutamic acid were found to supply 7.5% of the nitrogen required for the carbon being photosynthetically fixed at that time. The sum of the two amino acids concentrations represented 18% of the total dissolved organic nitrogen present.

3) Inorganic nitrogen is rapidly cycled through the particulate fraction and released as dissolved organic nitrogen. Within 48 hrs, 10% of the copper-chelex extractable dissolved organic nitrogen was derived from $K^{15}NO_3$ added to Auke Bay seawater.

4) Dissolved organic nitrogen is readily utilized as a carbon source below the euphotic zone. The dissolved organic nitrogen that accumulates from sinking cells is converted nearly quantitatively to ammonia. If subsequent increases in transparency of the overlying water allow phytoplankton growth to occur, the process is reversed and

part of the ammonia is converted to dissolved organic nitrogen, the remainder to the particulate fraction (i.e., phytoplankton).

5) During incorporation of a dissolved free amino acid molecule by phytoplankton, the assimilation of the absorbed carbon and nitrogen proceeded at differing rates. The nitrogen of glycine was found to be incorporated into phytoplankton cells nearly twice as fast as the carbon and the reverse order was found for glutamic acid. No explanation for this order was determined.

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APPENDIX

Appendix 1: Physical and chemical parameters of Auke Bay stations ON-2 to ON-27

Station ON-2 25 March 1968

Sky: overcast: Wind direction: 90° Weather: rain
Wind velocity: 7 knots Air temperature: 3°C

Depth	Salinity	Temp.	NO_3-N*	NH_3-N*	Dissolved organic N
0 m	30.00 °/₀₀	3.62°C	--	--	--
1	29.98	3.74	--	--	--
2	29.57	3.64	29.3	0.9	4.1
4	30.43	3.60	--	--	--
6	30.64	3.38	--	--	--
8	30.70	3.35	--	--	--
10	30.58	3.35	--	--	--
12	30.53	3.40	--	--	--
16	30.48	3.35	--	--	--
20	30.50	3.42	--	--	--
25	30.39	3.45	--	--	--
30	30.38	3.48	--	--	--
50	--	--	32.7	--	4.0

* ug-atom/liter

Station ON-3 28 March 1968

Sky: overcast Wind direction: 70° Weather: snow
Wind velocity: 13 knots Air temperature: 4°C

Depth	Salinity	Temp.	NO_3-N*	PO_4-P*	Si*
0 m	29.54°/$_{oo}$	3.52°C	31.4	2.31	--
1	29.54	3.52	--	--	--
2	29.54	3.52	31.4	2.26	--
4	29.61	3.52	--	--	--
5	--	--	31.4	2.35	64.8
6	29.58	3.52	--	--	--
8	29.58	3.52	--	--	--
10	29.65	3.54	31.4	2.28	--
12	29.60	3.60	--	--	--
15	--	--	31.6	2.37	64.0
16	29.72	3.61	--	--	--
20	29.82	3.60	--	--	--
25	29.90	3.60	32.0	2.37	--
30	29.81	3.72	--	--	--
35	--	--	32.9	2.40	--
45	--	--	33.5	2.46	--
50	--	--	34.3	2.78	--

* ug-atoms/liter

Station: ON-4 1 April 1968

Sky: clear Wind direction: 10° Weather: --
Wind velocity: 15 knots Air Temperature: 5°C

Depth	Salinity	Temp.	NO_3-N*	PO_4-P*	Ch-a+
0 m	29.79°/$_{oo}$	4.75°C	27.3	2.03	2
1	29.69	4.76	--	--	2
2	29.70	4.69	--	--	--
4	30.10	4.14	--	--	--
5	--	--	28.6	2.04	3
6	30.10	3.95	--	--	--
8	30.06	3.86	--	--	--
10	30.20	3.82	31.5	2.06	2.5
12	30.28	3.80	--	--	--
15	--	--	32.9	2.11	1
16	30.32	3.75	--	--	--
20	30.45	3.70	--	--	--
25	30.50	3.68	--	--	--
30	30.54	3.62	--	--	--
45	--	--	--	--	--
55	--	--	35.2	2.73	0.5

* ug-atoms/liter

+ arbitrary fluorescence units

Station ON-5 3 April 1968

Sky: light overcast Wind direction: -- Weather: --
Wind velocity: calm Air temperature: 4°C

Depth	Salinity	Temperature	Si (ug-at/liter)
0 m	30.03 °/₀₀	3.89°C	58.3
1	30.02	3.89	--
2	30.00	3.86	57.7
4	29.97	3.87	--
5	--	--	56.6
6	29.96	3.95	--
8	29.94	3.90	--
10	29.73	3.65	59.4
12	30.30	3.66	--
16	30.55	3.58	60.0
20	30.69	3.58	--
25	30.70	3.55	60.8
30	30.76	3.60	--
35	--	--	62.6
45	--	--	61.9
55	--	--	66.7

Station ON-5 (continued) 3 April 1968

Depth	NO_3-N*	PO_4-P*	NO_2-N*	Chl-a+
0 m	27	1.47	0.19	4
2	27	1.47	0.19	4
5	24	1.42	0.16	4
10	32	1.64	--	3
16	38	1.87	--	2
25	34	1.88	--	--
35	34	1.87	--	--
45	--	1.91	0.24	--
55	35	2.21	--	0.5

* ug-atoms/liter

+ arbitrary fluorescence units

Station ON-6 5 April 1968

Sky: heavy overcast Wind direction: -- Weather: --
Wind velocity: calm Air temperature: 4°C

Depth	Salinity	Temperature	Si (ug-at/liter
0 m	29.95°/₀₀	4.41	56.4
1	30.00	4.36	--
2	30.09	4.38	57.4
3	30.06	4.35	--
4	30.08	4.34	--
5	--	--	57.5
6	30.08	4.24	--
8	30.12	4.10	--
10	30.09	4.02	--
12	30.09	3.98	--
14	--	--	--
15	--	--	57.3
16	30.09	3.92	58.0
20	30.16	3.88	--
25	30.42	3.70	59.7
30	30.60	3.60	--
35	--	--	60.9
45	--	--	65.6

Station ON-6 (continued) 5 April 1968

Depth(m)	NO_3-N*	PO_4-P*	Reduced N*	Chl-a+
0	18.2	1.22	3.3	3
2	18.4	1.24	3.3	4
5	20.1	1.38	2.8	5
10	25.1	1.63	1.9	6
15	26.5	1.87	1.6	-
16	27.1	-	-	4
25	30.5	2.09	-	-
35	32.0	2.19	-	-
45	32.8	2.51	2.0	-

* ug-atoms/liter. Reduced N is the sum of ammonia and dissolved organic nitrogen

+ arbitrary fluorescence units

Station ON-7 8 April 1968

sky: heavy wind direction: -- weather: rain
wind velocity: calm air temperature: 4°C

Depth(m)	T(°C)	Salinity(°/₀₀)	Si(ug-at Si/liter)
0	4.55	29.92	50.4
1	4.65	30.15	-
2	4.49	30.20	55.2
4	4.38	30.14	-
5	-	-	53.5
6	4.44	30.35	-
8	4.62	30.14	-
10	4.44	30.42	55.5
12	4.30	30.46	-
16	4.10	30.54	61.2
20	3.92	30.62	62.1
25	3.70	30.88	-
30	3.60	31.10	-
35	-	-	61.5
45	-	-	62.1
55	-	-	66.3

Station ON-7 (continued), 8 April 1968

Depth(m)	NO_3-N*	PO_4-P*	Reduced N*	Chl-a+
0	6.9	0.70	4.7	24
2	10.6	1.00	3.3	20
5	10.6	1.02	3.5	20
10	15.4	1.30	3.4	18
16	23.4	1.94	1.0	5
20	26.8	2.21	-	3
35	28.1	2.26	-	-
45	29.9	2.42	3.8	-
55	30.3	2.54	-	< 0.5

* ug-atoms/liter. Reduced N is the sum of ammonia and dissolved organic nitrogen

+ arbitrary fluorescence units

Station ON-8 10 April 1968

Sky: overcast wind direction: 270° weather: snow
wind velocity: 5 knots air temperature: 0°C

Depth(m)	Salinity(°/₀₀)	T(°C)	Si(ug-at Si/liter)
0	30.10	4.10	47.5
1	30.20	4.14	-
2	30.18	4.15	48.0
4	30.35	4.13	-
5	-	-	48.0
6	30.48	4.14	-
8	30.55	4.14	-
10	30.52	4.12	48.2
12	30.60	4.08	-
14	30.58	4.05	-
16	30.59	4.06	53.6
20	30.61	4.07	56.8
25	30.68	4.01	57.3
30	30.92	3.86	-
35	-	-	61.4
45	-	-	62.6
55	-	-	62.8

Station ON-9 12 April 1968

Sky: overcast wind direction: 140° weather: snow showers
wind velocity: 20 knots air temperature: 1°C

Depth(m)	Salinity(°/₀₀)	T(°C)	Si(ug-at Si/liter)
0	31.19	3.66	52.2
1	31.09	3.68	-
2	31.10	3.78	47.8
5	31.05	3.80	48.9
8	31.06	3.82	-
10	-	-	50.0
12	31.14	3.82	-
16	-	-	49.4
20	31.46	3.62	55.8
25	-	-	57.9
45	-	-	-

Station ON-9 (continued) 12 April 1968

Depth(m)	NO_3-N*	PO_4-P*	Reduced N*	Chl-a+
0	13.1	1.43	6.5	16
2	12.8	1.50	5.0	16
5	13.1	1.51	7.1	16
10	16.8	1.78	-	14
16	-	1.57	5.5	17
20	23.8	2.19	3.9	5
25	28.4	2.52	-	1
45	29.4	2.62	3.8	< 0.5

* ug-atoms/liter. Reduced N is the sum of ammonia and dissolved organic nitrogen.

Light attenuation: percent incident radiation remaining

100%	0m
50%	1.5m
25%	2.1m
10%	4.0m
1%	9.4m

Station ON-10 15 April 1968

Sky: clear wind direction: -- weather: --
wind velocity: calm air temperature: 7°C

Depth(m)	Salinity(°/₀₀)	T(°C)	Si(ug-at Si/liter)
0	30.90	4.92	36.8
1	30.94	4.82	-
2	30.95	4.38	41.6
4	31.04	4.00	-
5	-	-	48.5
6	30.90	4.02	-
8	30.95	4.02	-
10	30.90	4.00	50.7
12	30.94	3.88	-
14	30.95	3.82	-
16	30.82	3.92	56.7
20	31.08	3.88	58.9
25	31.40	3.72	60.5
30	31.40	3.68	-
35	-	-	62.1
45	-	-	64.6
55	-	-	66.6

Station ON-10 (continued) 15 April 1970

Depth(m)	NO_3-N*	PO_4-P*	Reduced N*	Chl-a+
0	0.14	0.31	5.9	12
2	1.03	0.48	4.2	11
5	8.4	1.02	4.5	18
10	13.5	1.33	-	15
16	25.7	1.76	2.5	10
20	25.3	2.13	2.8	4
25	28.6	2.22	-	-
35	30.1	2.50	-	-
45	30.6	2.60	3.5	
55	34.5	2.69	-	< 0.5

* ug-atoms/liter. Reduced N is the sum of ammonia and dissolved organic nitrogen

+ arbitrary fluorescence units

Light attenuation (percent incident radiation remaining)

100%	0.0m
50%	0.9m
25%	1.9m
10%	3.4m
1%	8.6m

Station ON-11 19 April 1970

Sky: partial overcast Wind direction: 0° weather: --
wind velocity: 5 knots air temperature: 4°C

Depth (m)	Salinity (°/₀₀)	Temperature (°C)
0	30.88	4.05
1	30.93	4.03
2	31.12	4.00
4	31.39	4.00
6	31.45	3.99
8	31.43	3.98
10	31.43	3.98
12	31.42	3.98
16	31.42	3.94
20	31.42	3.89
25	31.40	3.89
30	31.32	3.92

Light attenuation: percent incident radiation remaining

100%	0.0m
50%	1.1m
25%	2.5m
10%	4.7m
1%	10.5m

Station PI-1 19 April 1968

Sky: partial overcast Wind direction: 0° weather: --
wind velocity: 5 knots air temperature: 4°C

Depth(m)	Salinity(°/₀₀)	T(°C)	Si(ug-at Si/liter)
0	31.35	4.46	-
2	31.22	4.40	59.8
4	31.30	4.25	-
5	-	-	61.5
8	31.36	4.28	-
10	31.45	4.22	61.5
12	31.38	4.30	-
16	31.22	4.14	61.7
20	31.42	4.10	60.2
25	31.25	4.16	60.8
30	31.28	3.90	60.4
45	-	-	62.1
100	-	-	64.1

Station PI-1 (continued) 19 April 1968

Depth(m)	NO_3-N*	PO_4-P*	Reduced N*	Chl-a+
0	25.7	2.02	4.3	3
2	26.3	2.00	2.9	2
5	26.8	2.12	5.1	1
10	30.0	2.08	-	2
16	31.0	2.09	3.6	3
20	27.3	2.03	4.5	2
25	27.6	2.08	-	-
35	28.7	2.19	-	-
45	29.4	2.22	6.0	-
100	31.9	2.39	-	-

* ug-atoms/liter. Reduced N is the sum of ammonia and dissolved organic nitrogen.

+ arbitrary fluorescence units

Station ON-12 22 April 1968

Sky: thin overcast wind direction: -- weather: --
wind velocity: calm air temperature: 7°C

Depth(m)	Salinity(°/₀₀)	T(°C)	Si(ug-at Si/liter)
0	30.60	5.16	23.0
1	30.50	5.30	-
2	30.68	4.97	24.0
4	31.04	4.70	-
5	-	-	42.8
6	31.12	4.42	-
8	31.35	4.19	-
10	31.34	4.00	52.6
12	31.30	3.98	-
16	31.45	3.90	56.0
20	31.40	3.84	57.6
25	31.40	3.78	59.7
30	31.30	3.80	-
35	-	-	60.8
45	-	-	61.8
55	-	-	61.6

Station ON-12 (continued) 22 April 1970

Depth(m)	NO_3-N*	PO_4-P*	Reduced N*	Chl-a+
0	0.10	0.24	4.3	9
2	0.35	0.26	4.6	16
5	11.3	1.15	4.2	11
10	21.5	1.82	2.8	5
16	24.4	2.05	3.7	3
20	25.4	1.96	-	2
25	30.7	2.23	-	-
35	29.0	2.44	-	-
45	29.5	2.47	4.2	-
55	30.2	2.41	-	-

* ug-atoms/liter. Reduced N is the sum of ammonia and dissolved organic nitrogen.

+ arbitrary fluorescence units

Light attenuation: percent incident radiation remaining

100%	0.0m
50%	0.9m
25%	1.5m
10%	3.5m
1%	10.5m

Station ON-13 24 April 1968

Sky: overcast wind direction: 110° weather: rain
wind velocity: 16 knots air temperature: 3°C

Depth(m)	Salinity(°/₀₀)	T(°C)	Si(ug-at Si/liter)
0	30.60	4.93	30.4
1	30.68	4.80	-
2	30.60	4.82	31.3
4	30.72	4.76	-
5	-	-	37.2
6	30.77	4.74	-
8	30.77	4.62	-
10	30.82	4.55	40.4
12	30.77	4.48	-
16	30.90	4.46	47.7
20	31.10	4.25	-
25	31.10	4.20	50.2
30	31.22	4.01	59.6
45	-	-	61.9
55	-	-	64.7

Station ON-13 (continued) 24 April 1968

Depth	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N	Chl-a
0	1.06	0.4	0.50	6.0	35
2	1.83	-	0.50	3.6	24
5	8.83	0.25	0.81	4.0	18
10	12.4	0.30	0.99	-	15
16	17.5	0.49	1.43	3.1	7
20	21.3	-	1.66	-	4
25	23.7	1.19	1.73	3.9	-
35	30.4	-	2.17	-	-
45	31.5	-	2.26	4.2	-
55	32.9	1.18	2.48	-	-

Light attenuation: percent incident radiation remaining

100%	0.0m
50%	1.2m
25%	2.0m
10%	3.5m
1%	9.6m

Station ON-14 26 April 1968

Sky: overcast wind direction: 130° weather: rain
wind velocity: 18 knots air temperature: 5°C

Depth(m)	Salinity(°/$_{oo}$)	T(°C)	Si(ug-at/liter)
0	30.62	5.02	34.7
1	30.60	4.98	-
2	30.55	5.00	34.9
4	30.59	4.95	-
5	-	-	32.0
6	30.64	4.90	-
8	30.68	4.86	-
10	30.69	4.85	33.8
12	30.69	4.80	-
16	30.73	4.78	51.9
20	30.82	4.38	61.0
25	30.85	4.21	61.2
30	31.12	3.94	-
35	-	-	62.3
45	-	-	65.2
55	-	-	66.7

Station ON-14 (continued) 26 April 1968

Depth	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N*	Chl-a+
0	4.72	0.43	0.52	5.4	21
2	2.91	0.39	0.44	5.4	18
5	3.33	0.32	0.45	5.4	22
10	4.17	0.35	0.59	8.3	24
16	16.1	0.95	1.39	5.4	6
20	-	-	1.92	-	2
25	23.4	0.94	1.98	-	2
35	27.1	-	2.02	-	-
45	26.8	-	2.17	1.5	-
55	27.7	1.81	2.21	-	-

* ug-atoms/liter

+ arbitrary fluorescence units

Light attenuation: percent incident radiation remaining

100%	0.0m
50%	0.5m
25%	1.9m
10%	2.9m
1%	10.8m

Station ON-15 29 April 1968

Sky: thin overcast wind direction: -- weather: --
wind velocity: calm air temperature: 7°C

Depth(m)	Salinity(°/₀₀)	T(°C)	Si(ug-at Si/liter)
0	29.80	5.62	11.5
1	30.12	5.63	-
2	30.52	5.12	12.3
4	30.60	4.62	-
5	-	-	36.5
6	30.64	4.56	-
8	30.75	4.37	-
10	30.78	4.25	53.7
12	30.64	4.25	-
16	30.74	4.12	61.0
20	31.00	3.92	62.1
25	31.44	3.84	61.2
30	30.71	3.78	-
35	-	-	64.5
45	-	-	64.5
55	-	-	67.7

Station ON-15 (continued) 29 April 1968

Depth(m)	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N*	Chl-a+
0	0.16	0.4	0.13	5.5	13
2	0.08	0.3	0.11	5.3	49
5	11.3	0.7	1.00	5.3	16
10	21.5	2.0	1.28	-	4
16	26.1	2.8	2.10	3.0	0
20	27.7	-	2.23	2.0	0.5
25	26.6	2.4	2.21	-	-
35	28.2	-	2.33	-	-
45	29.4	-	2.32	2.5	-
55	27.9	2.76	2.29	-	-

* ug-atoms/liter

+ arbitrary fluorescence units

Light attenuation: percent incident radiation remaining

100%	0.0m
50%	0.5m
25%	1.9m
10%	2.9m
1%	10.8m

Station ON-16 1 May 1968

Sky: partial overcast wind direction: 270° weather: --
wind velocity: 5 knots air temperature: 7°C

Depth(m)	Salinity(°/$_{oo}$)	T(°C)	Si(ug-at Si/liter)
0	29.55	6.45	-
1	29.52	6.10	-
2	29.92	6.04	16.6
4	30.28	6.00	-
5	-	-	26.9
6	30.36	5.60	-
8	30.74	5.03	-
10	30.68	5.09	33.0
12	31.02	4.78	-
16	31.22	4.49	49.9
20	31.35	4.04	56.5
25	31.44	4.19	59.3
30	31.18	4.01	-
35	-	-	62.0
45	-	-	62.9
55	-	-	62.9

Station ON-16 (continued) 1 May 1968

Depth	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N*	Chl-a+
0	0.04	0.6	0.12	4.3	12
2	0.04	0.5	0.16	4.3	24
5	3.39	0.6	0.49	4.2	24
10	7.16	0.6	0.72	-	13
16	8.24	2.9	1.66	0.3	4
20	23.8	3.2	1.92	0.4	2
25	26.0	4.3	2.15	-	-
35	26.7	-	2.34	-	-
45	-	-	2.43	0.7	-
55	28.0	3.8	2.34	-	-

* ug-atoms/liter

+ arbitrary fluorescence units

Station ON-17 3 May 1968

Sky: overcast wind direction: 180° weather: sleet
wind velocity: 10 knots air temperature: 2°C

Depth(m)	Salinity(°/$_{oo}$)	T(°C)	Si(ug-at Si/liter)
0	30.05	5.69	9.8
1	30.24	5.69	-
2	30.42	5.61	11.3
4	30.35	5.54	-
5	-	-	13.9
6	30.46	5.44	-
8	30.33	5.30	-
10	30.65	5.22	26.7
12	30.79	5.14	-
16	30.94	4.89	52.0
20	31.24	4.38	57.4
25	31.35	3.86	63.0
30	31.45	3.84	-
35	-	-	63.9
45	-	-	67.7
55	-	-	65.3

Station ON-17 (continued) 3 May 1968

Depth(m)	NO_3-N	NH_3-N	PO_4-P	Dissolved Organic N	Chl-a
0	0.04	0.6	0.10	4.2	31
2	0.15	0.4	0.08	3.2	40
5	0.81	0.5	0.19	2.9	32
10	4.66	0.5	0.58	-	18
16	20.4	2.8	1.67	2.3	2
20	-	-	2.02	1.8	2
25	27.9	2.4	2.44	-	0.5
35	27.3	-	2.49	-	-
45	30.0	-	2.56	-	-
55	28.3	3.5	2.58	-	-

Station ON-18 6 May 1968

Sky: thin overcast wind direction: 90 weather: --
wind velocity: 5 knots air temperature: 7°

Depth(m)	Salinity(°/$_{oo}$)	T(°C)	Si(ug-at Si/liter)
0	29.26	7.25	6.0
1	29.85	7.34	-
2	29.80	7.40	6.3
4	30.82	5.70	-
5	-	-	27.1
6	20.88	5.17	-
8	31.15	4.94	-
10	31.26	4.86	48.6
12	31.58	4.68	-
16	31.97	4.05	63.9
20	31.88	4.08	69.3
25	32.01	3.90	69.3
30	31.65	4.05	-
35	-	-	67.3
45	-	-	66.6
55	-	-	71.4

Station ON-18 (continued) 6 May 1968

Depth(m)	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N*	Chl-a+
0	0.70	0.5	0.10	3.6	9
2	0.58	0.7	0.22	5.2	17
5	8.36	0.6	0.92	2.5	20
10	17.9	1.0	1.93	-	4
16	26.5	3.3	2.60	1.5	1
20	24.1	-	2.91	-	<0.5
25	28.9	4.6	2.85	-	-
35	29.5	-	2.81	-	-
45	28.8	-	2.98	1.0	-
55	29.1	3.5	3.12	-	-

* ug-atoms/liter

+ arbitrary fluorescence units

Station ON-19 8 May 1970

Depth(m)	Salinity(°/$_{oo}$)	T(°C)	Si(ug-at Si/liter)
0	29.02	7.58	6.3
1	29.20	7.33	-
2	29.16	7.76	6.6
4	30.24	7.46	-
5	-	-	6.6
6	30.38	6.09	-
8	30.62	5.80	-
10	30.65	5.61	49.8
12	30.68	5.11	-
16	31.32	4.55	64.9
20	31.58	4.34	75.5
25	32.05	4.05	-
30	31.58	3.98	-

Station ON-19 (continued) 8 May 1970

Depth(m)*	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N*	Chl-a+
0	0.04	0.5	0.09	4.2	4
2	0.04	0.3	0.05	4.6	8
5	0.22	0.8	0.08	3.8	13
10	15.8	1.9	0.82	2.3	8
16	19.8	2.6	0.76	3.8	5
20	25.4	2.9	1.19	3.3	1

* ug-atoms/liter

+ arbitrary fluorescence units

Station ON-20 10 May 1968

Depth(m)	Salinity(°/$_{oo}$)	T(°C)	Si(ug-at Si/liter)
0	29.86	8.75	3.14
1	29.82	8.75	-
2	30.36	8.00	2.73
4	30.60	7.41	-
5	-	-	5.80
6	30.92	6.52	-
8	30.73	6.02	-
10	30.92	5.79	24.2
12	31.12	5.60	-
16	31.95	4.35	63.5
20	32.13	4.00	60.1
25	32.12	4.16	68.0
30	31.45	4.16	-
35	-	-	65.7
45	-	-	63.9
55	-	-	66.6

Station ON-20 (continued) 10 May 1968

Depth(m)	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N*	Chl-a+
0	0.00	1.0	0.13	4.7	3
2	0.00	0.7	0.10	4.7	3
5	0.29	0.9	0.23	6.0	18
10	11.7	0.8	0.71	2.1	30
16	27.9	3.9	2.38	0.4	2
20	26.8	-	2.21	1.2	4
25	30.6	4.3	1.74	-	-
35	29.7	-	2.56	-	-
45	29.7	-	2.61	-	-
55	29.7	4.7	2.65	-	-

* ug-atoms/liter

+ arbitrary fluorescence units

Light attenuation: percent incident remaining

100%	0.0m
50%	1.5m
25%	3.5m
10%	5.4m
1%	7.8m

Station ON-21 13 May 1968

Sky: clear wind direction: 290° weather: --
wind velocity: 2 knots air temperature: 13°C

Depth(m)	Salinity(°/$_{oo}$)	T(°C)	Si(ug-at Si/liter)
0	29.00	10.02	6.0
1	29.92	9.26	-
2	30.09	8.32	5.7
4	30.82	6.00	-
5	-	-	7.4
6	31.32	5.19	-
8	31.56	4.55	-
10	31.60	4.32	49.1
12	31.72	4.06	-
16	31.82	3.90	57.4
20	31.88	3.93	63.8
25	31.72	3.84	67.8
30	31.84	3.94	-
35	-	-	65.9
45	-	-	67.2
55	-	-	67.8

Station ON-21 (continued) 13 May 1968

Depth(m)	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N*	Chl-a+
0	0.07	0.5	0.05	5.7	0.5
2	0.00	0.5	0.03	4.6	4
5	0.07	0.5	0.08	6.4	14
10	24.5	1.4	1.70	-	13
16	27.9	3.8	2.06	0.6	5
20	30.5	4.2	2.27	-	0.5
25	20.9	4.0	-	1.0	-
35	30.3	-	2.19	-	-
45	30.4	-	2.33	0.4	-
55	30.4	4.2	2.43	-	-

* ug-atoms/liter

+ arbitrary fluorescence units

Station ON-22 16 May 1968

Sky: overcast wind direction: 140° weather: rain
wind velocity: 5 knots air temperature: 9°C

Depth(m)	Salinity(°/₀₀)	T(°C)	Si(ug-at Si/liter)
0	29.68	9.18	3.32
1	29.85	8.78	-
2	30.28	8.04	4.54
4	30.48	7.42	-
5	-	-	3.84
6	30.78	7.12	-
8	31.12	6.70	-
10	31.10	6.40	5.12
12	31.15	5.36	-
16	31.35	4.50	16.0
20	31.58	4.12	44.9
25	31.65	4.32	57.5
30	31.52	4.18	-
35	-	-	61.9
45	-	-	63.2
55	-	-	61.9

Station ON-22 (continued) 16 May 1968

Depth(m)	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N*	Chl-a+
0	0.07	0.5	0.04	3.9	9
2	0.00	0.6	0.09	4.7	11
5	0.07	0.5	0.04	4.9	19
10	0.15	0.7	0.08	-	18
16	10.2	0.8	0.79	2.6	46
20	23.9	4.9	1.80	0.0	16
25	29.1	-	2.27	-	4
35	31.5	-	2.43	0.0	5
45	31.5	4.4	2.44	-	0
55	31.5	-	2.41	-	-

* ug-atoms/liter

+ arbitrary fluorescence units

Station PI-2 13 May 1968

Depth(m)	Salinity(°/₀₀)	T(°C)	Si(ug-at Si/liter)
0	30.17	8.30	16.8
2	30.88	7.72	-
4	31.24	6.10	-
5	-	-	-
6	31.45	5.90	28.8
10	31.55	5.28	15.1
15	31.62	5.04	34.7
20	31.88	4.90	-
25	32.02	4.82	-
30	32.10	4.72	44.7

Station PI-2 (continued) 13 May 1968

Depth(m)	NO_3-N*	NH_3-N*	PO_4-P*	Chl-a+
0	5.38	1.4	0.46	3
5	4.43	0.7	0.37	6
10	12.6	0.7	0.88	4
15	16.2	1.3	1.14	7
30	21.2	1.3	1.50	4

* ug-atoms/liter

+ arbitrary fluorescence units

Station ON-23 23 May 1968

Sky: overcast wind direction: 140° weather: rain
wind velocity: 13 knots air temperature: 7°C

Depth(m)	Salinity(°/₀₀)	T(°C)	Si(ug-at Si/liter)
0	19.35	11.40	12.1
1	21.76	11.46	-
2	22.14	11.08	11.0
4	27.17	11.22	-
5	-	-	6.95
6	29.87	9.47	-
8	30.60	7.66	-
10	31.15	5.94	5.80
12	31.56	5.48	-
16	31.56	5.20	7.98
20	31.93	4.70	14.5
25	32.02	4.35	-
30	31.80	4.28	-
45	-	-	27.0

Station ON-23 (continued) 23 May 1968

Depth(m)	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N*	Chl-a+
0	0.15	0.41	0.14	6.4	20
2	0.07	0.45	0.09	6.0	20
5	0.58	1.2	0.16	5.6	6
10	0.07	0.9	0.43	6.6	10
16	2.86	0.7	0.15	4.3	14
20	9.4	0.6	0.57	-	12
45	27.3	-	2.46	2.5	3

* ug-atoms/liter

+ arbitrary fluorescence units

Station ON-24 27 May 1968

Depth(m)	Salinity(°/₀₀)	T(°C)	Si(ug-at Si/liter)
0	20.10	11.03	6.58
1	20.38	11.05	-
2	20.62	10.30	6.25
4	22.32	9.98	-
5	-	-	5.22
6	25.44	9.10	-
8	27.52	9.58	-
10	29.22	8.89	5.54
12	30.68	7.54	-
16	31.42	5.92	11.5
20	31.48	5.35	7.86
25	31.46	4.98	23.0
30	31.50	4.50	-
35	-	-	58.3
45	-	-	62.7
55	-	-	60.7

Station ON-24 (continued) 27 May 1968

Depth(m)	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N*	Chl-a+
0	0.07	0.9	0.78	5.9	12
2	0.07	0.8	0.65	5.4	11
5	0.03	1.0	0.83	5.3	14
10	1.59	1.1	1.71	-	5
16	17.0	3.0	1.34	3.4	4
20	19.2	3.6	1.60	1.5	-
25	16.7	3.0	1.49	-	4
35	29.6	-	2.69	-	-
45	30.8	5.5	2.80	0.0	-
55	30.5	-	2.82	-	-

* ug-atoms/liter

+ arbitrary fluorescence units

Station ON-25 29 May 1968

Depth(m)	Salinity(°/₀₀)	T(°C)	Si(ug-at Si/liter)
0	21.66	12.42	5.29
1	21.78	12.56	-
2	22.52	12.48	4.58
4	25.66	9.85	-
5	-	-	4.39
6	27.90	8.92	-
8	29.86	8.35	-
10	30.52	7.32	7.10
12	30.92	7.22	-
16	31.44	6.08	18.0
20	31.72	5.50	16.5
25	31.94	4.82	26.0
30	31.88	4.52	-
35	-	-	24.8
45	-	-	61.0

Station ON-25 (continued) 29 May 1968

Depth(m)	NO_3N*	NH_3-N*	PO_4-P*	Dissolved Organic N*	Chl-a+
0	0.04	1.5	0.06	4.6	8
2	0.04	0.9	0.07	6.3	8
5	0.37	1.2	0.08	5.5	12
10	2.80	1.7	0.22	-	6
16	7.62	1.9	0.56	3.4	5
20	14.7	3.1	1.53	2.4	2
25	15.8	3.2	1.39	-	2.5
45	29.7	4.7	2.60	1.6	2

* ug-atoms/liter

+ arbitrary fluorescence units

Station ON-26 31 May 1968

Depth(m)	Salinity(°/$_{oo}$)	T(°C)	Chl-a^{+}
0	21.83	12.10	5
1	21.66	12.13	-
2	21.77	12.13	6
4	22.04	11.88	-
5	-	-	11
6	25.52	10.08	-
8	27.76	9.06	-
10	29.62	7.97	9
12	29.78	7.84	-
16	31.22	6.34	4
20	31.56	5.64	1
25	31.58	4.96	1
30	31.72	4.52	-

+ arbitrary fluorescence units

Station ON-26 (continued) 31 May 1968

Depth(m)	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N*
0	0.00	1.1	0.11	3.9
2	0.15	1.1	0.16	4.7
5	0.15	1.1	0.14	3.6
10	1.91	1.5	0.27	-
16	8.66	3.2	0.87	2.0
20	14.6	3.4	1.36	2.0
25	21.4	4.8	2.19	-
35	28.3	-	2.93	-
45	31.4	5.3	3.37	0.0
55	31.2	5.6	3.38	-

* ug-atoms/liter

Station ON-27 3 June 1968

Sky: overcast wind direction: 130° weather: rain
wind velocity: 13 knots air temperature: 8°C

Depth(m)	Salinity(°/₀₀)	T(°C)	Chl-a+
0	21.90	11.60	4
1	21.92	11.65	-
2	22.00	11.58	4
4	23.52	10.80	-
5	-	-	4
6	26.92	9.56	-
8	29.00	8.44	-
10	30.30	7.32	2
12	30.98	6.72	-
16	31.58	5.38	2
20	31.78	4.86	2
25	31.76	4.52	1
30	31.88	4.26	-

+ arbitrary fluorescence units

Station ON-27 (continued) 3 June 1968

Depth(m)	NO_3-N*	NH_3-N*	PO_4-P*	Dissolved Organic N*
0	0.70	1.3	0.17	5.0
2	0.00	1.3	0.20	4.4
5	0.00	1.2	0.19	4.0
10	5.07	1.7	0.61	-
16	10.1	4.5	1.54	4.2
20	20.6	-	2.46	0.0
25	24.4	7.6	2.55	-
35	28.6	-	3.57	-
45	24.8	5.6	2.92	0.0
55	28.4	6.6	3.14	-

* µg-atoms/liter